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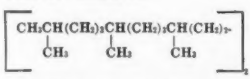

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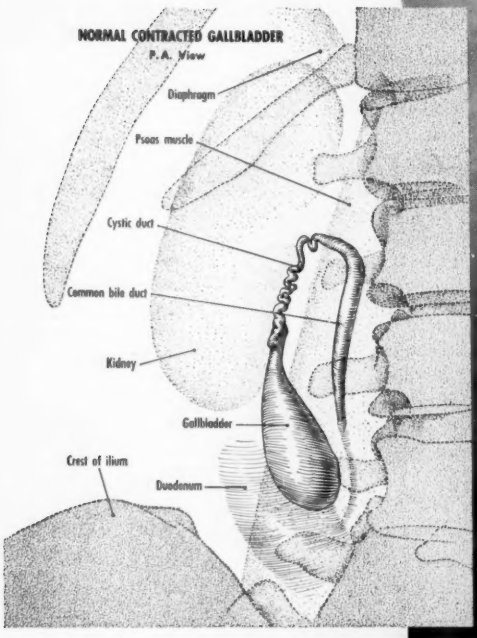

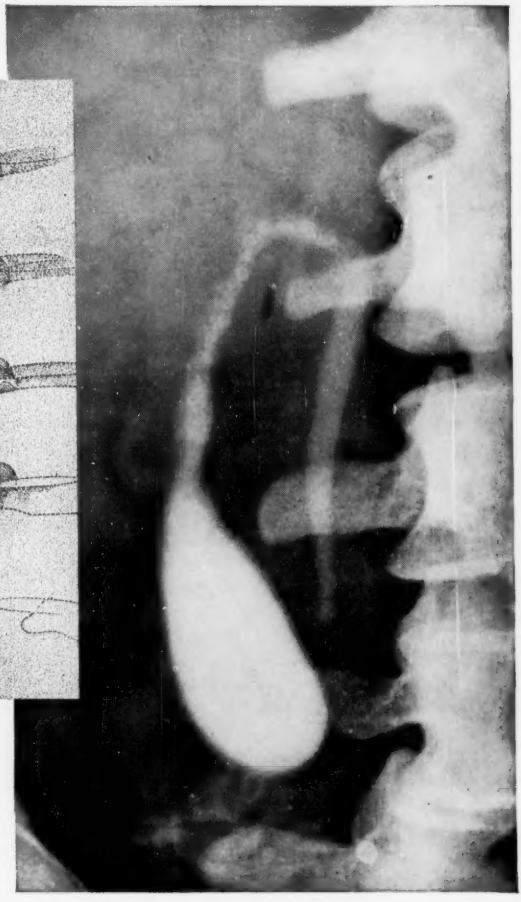

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MARTHE VOGT*

Department of Pharmacology,
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THE EFFECTS OF HEXOESTROL AND OF "AMPHENONE B" ON MORPHOLOGY AND FUNCTION OF THE RAT ADRENAL CORTEX

This investigation forms part of a study of the secretory capacity of the rat's adrenal cortex after structural changes have been produced in the gland by the administration of drugs and hormones. The two examples to be discussed are the effects of hexoestrol[21] and of 'amphenone B' (1, 2-bis (p-aminophenyl)-2 methyl-propanone-1 dihydrochloride). The latter substance, subsequently to be called 'amphenone,' was generously supplied by Dr. R. Hertz.

The functional state of the glands was tested by collecting the venous effluent from the left adrenal gland during anaesthesia and then carrying out a chemical estimation of the corticosterone secreted; unlike other species, the rat does not produce cortisol,[4] even after intensive treatment with ACTH[18]; in this respect the rat differs from the rabbit which has been shown to secrete cortisol after prolonged administration of ACTH.[24] Since, again in contrast to the rabbit, the rat does not secrete dehydrocorticosterone either, corticosterone estimations are a reliable guide to the rat's secretion of 'glucocorticoids.'

The conditions of the experiments were those of operative stress, and the secretion was therefore approaching or equalling maximal secretory capacity of the gland. This is shown by the fact that infusion of ACTH during such an operation produces little further increase in hormone output. Information on 'resting secretion' is not afforded by this technique.

METHODS

Adult male rats weighing between 250 and 400 g were used. The pre-operative treatment consisted of either:

(a) Daily injections of 0.3-0.5, and usually 0.4 mg hexoestrol/kg, given subcutaneously as 0.2 per cent solution in oil, the number of injections varying between 1 and 15.

(b) Daily administration, by stomach tube, of 0.2 g amphenone/kg as 5 per cent solution in water. The solutions were freshly prepared every 2 or 3 days, and stored in the refrigerator.

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* Reader, Department of Pharmacology.

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(c) Four daily injections of hexoestrol as under (a), followed by at least four days in which treatments (a) and (b) were combined.

Estimation of corticosterone

The method[21] consisted of collecting blood from the left renal vein in a heparinized rat anaesthetized either with urethane (1.5 g/kg) or pentobarbital (45 mg/kg). Tributaries to the renal vein other than the adrenal vein were tied, and so was the entry of the renal vein into the vena cava. The right femoral vein was cannulated so that intravenous injections could be given, and the blood pressure was recorded from the right femoral artery whenever desirable.

The blood was centrifuged, the plasma extracted with organic solvents and the purified extract chromatographed on paper with one of Bush's[8] solvent systems. Markers of pure steroids were run together with the extracts. After developing the chromatogram, the steroids were located by U.V. absorption, and the corticosterone was eluted with ethanol. The eluate was evaporated to dryness and the colour formed by the residue on incubation with blue tetrazolium measured in the spectro-photometer at 520 mμ (modification of the method of Mader and Buck[16]). The possible occurrence of substances other than corticosterone was investigated by spraying developed chromatograms with a 12 per cent solution of NaOH in water containing 3 mg blue tetrazolium per 100 ml, drying the paper rapidly and examining the fluorescence in U.V. light.[8]

RESULTS

Hexoestrol. It is well known that oestrogens in general, and stilboestrol and hexoestrol in particular, cause a rapid loss of lipids from the adrenal cortex[15, 20]; the effect is dependent on the integrity of the pituitary and persists for weeks provided treatment is maintained; only after many months may there be restitution of normal lipid content.[16a] The loss of lipids is accompanied by a variable hypertrophy of the gland and an increase in vascularity which may lead to hemorrhage; following oestradiol administration, increased amounts of ACTH are circulating in the blood.[6] The functional state of such adrenals has been a matter of much speculation, hyperactivity being stipulated by some, exhaustion following initial hyperactivity by others; a third view[20] suggested that the hypertrophy might be a secondary effect of excessive ACTH secretion, resulting from lack of hormone production by an inhibited adrenal cortex. This latter hypothesis would create an analogy between the action of thiouracil on the thyroid and that of oestrogens on the adrenal.

Figure 1 represents the results of a group of experiments carried out in urethane anaesthesia (left part of the figure), and another group done under pentobarbital (right part). The secretion per min per g adrenal is shown in black columns, and the value per single adrenal per hr per kg body weight in white columns. After hexoestrol the secretion was severely

depressed, both per g adrenal tissue and per kg body weight. Whereas the secretion of normal rats was the same with both anaesthetics, the depression by hexoestrol was more severe in urethane anaesthesia. In these experiments, the shortest treatment with hexoestrol lasted 3, and the longest, 15 days. The depression obtained was approximately the same for all durations.

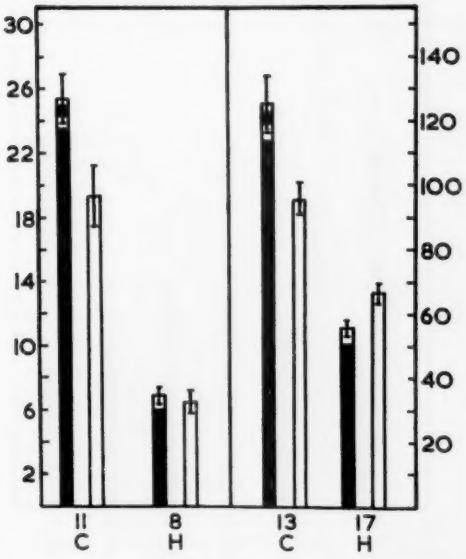

FIG. 1. Corticosterone secretion of rats, means and standard errors of the mean.
Left part: urethane anaesthesia.
Right part: pentobarbital anaesthesia.
Left ordinate and black columns: μg corticosterone/g adrenal/min.
Right ordinate and white columns: μg corticosterone/adrenal/hr/kg bodyweight.
Number of rats under each pair of columns.
C: normal controls.
H: rats given hexoestrol, 0.4 mg/kg subcutaneously for 3-15 days.

In order to test the hypothesis that the fall in secretion was due to 'exhaustion by initial stimulation,' shorter experimental periods were tried. When two injections were given, depression was slightly less than before. The experiments were then further shortened: intervals of 24, 4½, and 3½ hours were allowed between injection of hexoestrol and cannulation of the renal vein. In no instance was the secretion greater than normal, and in two of the experiments depression had already set in.

When the injections of hexoestrol were allowed to lapse for a week, recovery of secretory performance had taken place.

Experiments were also carried out to see whether the diminished secretion of corticosterone was indicative of genuine depression of synthesis or whether another steroid was produced which might replace the normal compound. This was done by carrying out the NaOH-fluorescence test on chromatograms of plasma from rats treated with hexoestrol; any steroids with an α,β-unsaturated ketone group are shown up by this reaction.[5] Not only was there no new compound to be found in such chromatograms, but the decrease in secretion of corticosterone was accom-

panied by a decreased production of the androgen normally found in rat adrenal blood.

Ways were then sought to antagonize the depression of hormone synthesis exerted by hexoestrol.[18] In one type of experiment, the immediate effect of various infusions was tried (Fig. 2). Rats were injected with hexoestrol for periods of 4 or 5 days, and a first 15-minute sample of blood collected in the usual way from the left adrenal. Then 8 ml of different solutions were infused, 4 ml during the 10 minutes before, and 4 ml during the first 10 minutes of collecting a second 15-minute sample of adrenal blood. Sometimes collection of the pre-infusion sample was omitted. When the solution infused was plasma from normal rats, corticosterone secretion per g adrenal reverted to normal figures (Fig. 2, column 3). If infusion was carried out with the same amount of plasma taken from rats injected with hexoestrol for five days, no improvement in secretory performance ensued. This result pointed to the absence of a normal plasma constituent from the blood of rats injected with hexoestrol as a possible cause of the functional deficiency of the adrenal cortex in these animals.

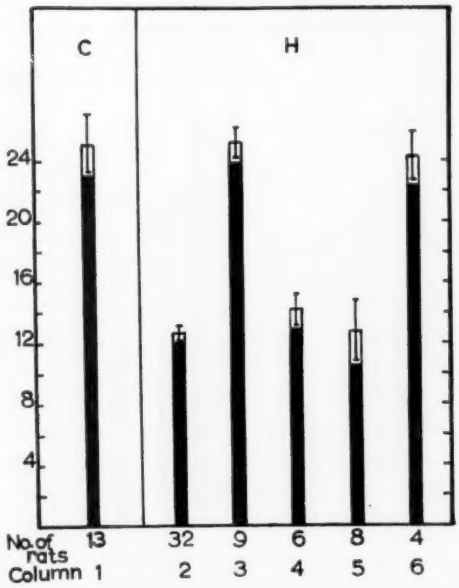

FIG. 2. Corticosterone secretion of rats, means and standard errors of the mean.
Ordinate: μg corticosterone/g adrenal/min.
Column 1: normal rats.
Column 2: rats given hexoestrol, 0.4 mg/kg subcutaneously for 4-6 days.
Columns 3-6: all rats had 4-5 injections of hexoestrol (0.4 mg/kg subcutaneously) and in addition:
Column 3: an infusion of 8 ml plasma from normal rats.
Column 4: an infusion of 8 ml plasma from hexoestrol-pretreated rats (0.4 mg/kg for 5 days).
Column 5: an infusion of 8 ml 5% bovine albumin.
Column 6: amphenone (0.2 g/kg for 4 days by stomach tube), while hexoestrol injections were being continued.

The possibility, however, existed that the beneficial effect of normal plasma was due to a mere dilution of the hexoestrol present in the tissues; this was ruled out by infusing another hexoestrol-free solution of about the same colloid-osmotic pressure as plasma, a 5 per cent solution of bovine albumin. Such infusions had the same effect on the blood pressure as had rat plasma, but were devoid of any restorative action on cortical secretion (Fig. 2, column 5). It may be concluded from these experiments that hexoestrol does not produce its inhibitory effect on cortical secretion by causing any profound damage to the enzyme systems of the cell, but by depriving the adrenal of some substance which can be supplied by a brief infusion of normal rat plasma.

In the search for the substance which is lacking in adrenals of rats given hexoestrol, cholesterol suggests itself as a possibility. When hexoestrol is administered, there is rapid loss from the cortex of sudanophilic material, of which cholesterol is a major component; depletion persists as long as the hexoestrol is given,[16] in contrast to what happens after administration of ACTH or in prolonged stress, where there is usually only an initial fall followed by an accumulation of cholesterol. Plasma cholesterol is very low after hexoestrol administration, and a lack of this precursor of adrenal steroids in the blood would explain the beneficial effects of infusing normal plasma to hexoestrol-treated rats, and the lack of improvement when plasma from rats which have themselves had hexoestrol is given instead. According to this theory, the damaging action of hexoestrol on the rat adrenal is exerted through an interference with cholesterol synthesis, leading to a decrease in the available amount of a material necessary for steroid synthesis. Direct support for this interpretation was obtained by Boyd and McGuire[3] who demonstrated a decrease in cholesterol synthesis from C^{14}-labelled acetate in liver slices of rats given hexoestrol for periods of 6-13 days.

Other effects of hexoestrol can now be interpreted as a result of the interference with cholesterol synthesis. There will be increased production of ACTH due to a lack of circulating corticosterone; this leads to hypertrophy and hyperplasia of the adrenal cortex, and to hypertrophy of the anterior lobe of the pituitary, if treatment is sufficiently prolonged. The condition would thus be analogous to the response of the thyroid when antithyroid drugs are administered.

An important question is whether this action of hexoestrol occurs in other species including man or is restricted to the rat. Experiments on cats and rabbits have shown that the cat is very intolerant to hexoestrol and reacts with liver damage to rather small doses; adrenal changes were not

produced with doses compatible with survival.[18] The rabbit, however, tolerated large amounts of hexoestrol, but neither adrenal lipids, nor corticosterone and cortisol secretion measured in operative stress, were decreased.[21]

In man, there is as yet no evidence that adrenal histology or secretion are affected, though influences of oestrogens on plasma cholesterol and its transport mechanisms have been reported.[1, 16b] It is also of some interest to

TABLE 1. CORTISONE SECRETION OF MALE RATS GIVEN AMPHENONE (0.2 G/KG) DAILY BY STOMACH TUBE

	Body weight (g)			Adrenal	Secretion of corticosterone	
No.	At start	At end	Days treated	weight (mg/kg b.w.)	μg/g gland/min	μg/gland/hr/kg b.w.
1	406	398	10	72	32.0	137
2	330	308	12	109	40.3	264
3	330	300	12	114	40.7	278
4	334	285	14	149	29.4	275
5	382	354	14	83	19.1	95
6	320	279	17	145	19.8	173
7	316	274	17	117	28.2	199
Mean 1–7		314	10–17	113* ± 11†	29.9 ± 2.9†	203* ± 26†
8	265	247	2	99	30.3	180
9	295	285	2	89	34.2	183
10	291	283	4	93	42.8	239
11	270	266	4	104	39.7	248
12	280	266	4	120	34.8	251
Mean 10-12		272	4	106* ± 8†	39.1* ± 2.4†	246* ± 4†
Mean 13-25 (Controls)	338		0	64 ± 2†	25.1 ± 1.8†	95 ± 5†

* Significantly different from controls.
† Standard error of the mean.

know whether all oestrogens act like hexoestrol. The answer to this question[13] is that the differences between oestrogens are quantitative, but not qualitative.

Amphenone. In the first experiments, dosage and duration of treatment was that recommended by Hertz *et al.*[10] It is clear from Table 1 that adrenal hypertrophy was considerable after 10-17 days' feeding of amphenone; the corticosterone secretion per g gland was high, but still within normal limits, and, owing to the enlargement of the gland, the secretion per single adrenal and per kg bodyweight was greatly increased. This result was very sur-

prising, particularly in view of the fact that, in man, amphenone has been reported[9] to decrease the excretion of 17-hydroxycorticoids in the urine and the level of 17-hydroxycorticoids in the plasma. In these patients, however, treatment was never longer than two or five days, and therefore a series of experiments of short duration was carried out in the rat.

Table 1 shows that the results were essentially the same as with the chronic treatment. After two days, adrenal size was already increased, secretion per g gland was on the upper limit of normal, and secretion per kg bodyweight significantly above normal. After four days, all these effects had progressed further, and even secretion per g gland had risen signifi-

TABLE 2. CORTISONE SECRETION OF MALE RATS BEFORE AND AFTER INTRAVENOUS INFUSION OF AMPHENONE

No.	*Body weight (g)*	*Dose infused (mg/kg)*	*Duration of infusion (min)*	*μg corticosterone secreted per g gland per min*		*Reduction in secretion (%)*
				Before infusion	*After infusion*	
1	325	50	8	32.0	13.6	58
2	318	63	7	26.4	14.5	45
3	330	58	3	37.2	23.4	37

cantly above the normal range. From this material it cannot be decided whether there is actually a peak in the effects after a few days with some recovery towards normal if treatment is prolonged; it is more likely that the peak at four days was only accidental. Histologically, accumulation of lipids and hypertrophy are noticeable after two days and go on increasing as treatment is being continued.

These experiments do not give any information on corticoid secretion in the unstressed state, but they show conclusively that the adrenal has a much greater secretory capacity during stress when the rat has been fed amphenone for 2 to 17 days than without that treatment.

Two groups of authors[7, 11] had come to a similar conclusion by attempting to carry out tests of adrenal function in rats fed with amphenone. Though interpretation was difficult, the experiments suggested an increased rather than a decreased capacity for secretion in these animals.

Inhibition of steroid synthesis has been reported when amphenone is infused intravenously in the hypophysectomized dog[8] or added to the perfusion fluid of calf adrenals.[17] In the dogs, the response to an intravenous injection of ACTH was inhibited, and in the calves, synthesis of cortico-

sterone, cortisone, and cortisol was depressed, irrespective of whether ACTH had been added to the perfusion fluid. In an attempt at finding out whether the species or the mode of administration was responsible for the different responses, amphenone was given intravenously to rats, one sample of blood being collected before and one after the infusion of amphenone. The dose was the same as that used in the hypophysectomized dogs.*

TABLE 3. CORTICOSTERONE SECRETION OF MALE RATS INJECTED FOR FOUR DAYS WITH HEXOESTROL (0.4 MG/KG) AND THEN GIVEN AMPHENONE (0.2 G/KG) BY MOUTH AND HEXOESTROL (0.4 MG/KG) SUBCUTANEOUSLY

No.	Body weight (g)		Days of combined treatment	Adrenal weight (mg/kg b.w.)	Secretion of corticosterone	
	At start	At end			μg/g gland/min	μg/gland/hr/kg b.w.
1	356	340	4	90	24.5	132
2	315	295	4	116	20.2	140
3	414	352	4	89	28.3	151
4	335	303	4	100	24.1	144
5	329	260	9	154	13.3	123
6	358	280	9	110	19.3	126
7	370	282	10	106	24.2	153
8	345	268	10	115	16.2	112
9	332	263	11	133	13.6	108
10	324	270	11	96	29.2	169
11	362	287	12	176	21.2	224
Mean 12-43*	(Controls*)	323	0	91.5	12.7 ± 0.46	68.6 ± 6.0

* Thirty-two rats which had hexoestrol (0.4 mg/kg) subcutaneously for 4-6 days.

The results are summarised in Table 2. In contrast to the experiments in which amphenone was fed, corticosterone secretion was reduced to about one-half by approximately 50 mg/kg intravenous amphenone. It thus appears that not the species but the mode of administration was responsible for the inhibition of steroid synthesis experienced in the one and the stimulation experienced in the other type of experiment. It is of some interest to note that the intravenous amphenone was very poorly tolerated by the rats, and that infusion of large volumes of 0.9 per cent NaCl solution was required to keep the rats alive and their blood pressure at a safe level. A fall in blood pressure is also reported by Thorn *et al.*[39] in a patient who was given amphenone by the mouth. Whether the inhibition following intravenous amphenone is only a result of overdosage was checked by repeating the experiments with smaller amounts. Four infusions were carried out,

and the doses of amphenone used were 3.3, 5, 10, and 11 mg/kg. The blood pressure was kept steady by infusion of rat blood whenever required. Small decreases in secretion of between 10 per cent and 15 per cent were observed, changes which hardly exceed accidental variations. There did not appear to exist a dose range at which increased secretion, as seen in the feeding experiments, occurred.

Since the effects of hexoestrol on corticosterone production and lipid storage in the adrenal are the opposite of those of amphenone, an attempt was made to restore the secretory depression and lipid loss due to hexoestrol by administering amphenone. All rats were first given four injections of hexoestrol, a treatment of sufficient duration to cause loss of lipids from all cortical layers except the glomerulosa and severe depression of secretory capacity. The hexoestrol injections were then combined with daily oral doses of amphenone for periods ranging from 4-12 days (Table 3). In all these rats, lipids were deposited in the fasciculata so that the glands would have appeared like normal glands, had it not been for the hypertrophy and for the enlarged blood vessels in the zona reticularis. Secretion was restored to normal in all rats in which the combined treatment did not exceed four days (see also column 6, Fig. 2), whereas longer duration of the combined treatment led to normal figures per g gland in four and subnormal figures in three rats. It is, however, obvious that even in those animals in which secretion per g adrenal did not attain normal figures, secretion per kg bodyweight was far above that of rats to which hexoestrol alone had been administered. A striking feature of the combined treatment with hexoestrol and amphenone was that it invariably produced jaundice, whereas jaundice was not observed in rats given either compound alone.

Any attempt at interpreting the mode of action of amphenone will have to take into consideration the effects of ACTH on adrenal secretory capacity and on cortical morphology. The release of ACTH by amphenone cannot, as with hexoestrol, be interpreted as due to low levels of circulating cortical hormone, but is probably a consequence of the toxicity of the compound. There can, however, be no doubt about its release; it is suggested by the hypertrophy of the gland, its increase in vascularity and the demonstration by Hertz and Tullner[9] that hypophysectomized, ACTH-maintained rats do not exhibit adrenal changes when fed with amphenone.

On comparing the effects of four days' feeding with amphenone and four days' injections of ACTH[18] (12 hourly injections of 1 u. Cortrophin Z, Organon, a 'delayed action-ACTH,' subcutaneously), lipid accumulation was found to be about the same, whether ACTH or amphenone had been given. When treatment was prolonged, lipid deposition became more excessive with amphenone than with ACTH. In the experiments which lasted

four days, ACTH caused an increase in corticosterone secretion (per kg bodyweight) of approximately 50 per cent, whereas amphenone caused an increase of 150 per cent. Since, in such short experiments, changes in dosage and timing of the ACTH injections did not appear to increase the secretory performance any further, one might suspect an effect of amphenone on adrenal metabolism in addition to its capacity of releasing ACTH. *In vitro* effects have so far only been described as inhibitory,[5,17] but the possibility that smaller doses might stimulate some phase of synthesis has not yet been explored.

SUMMARY

Adrenal secretory capacity was measured in rats by estimating the corticosterone secreted by the left adrenal during operative stress after the production of lipid depletion by hexoestrol and of lipid accumulation by amphenone.

Hexoestrol (0.4 mg/kg subcutaneously) inhibits corticosterone secretion, and this effect begins after as short an administration as two days and persists as long as hexoestrol is given. It is completely reversible by:

(a) Stopping the treatment

(b) An intravenous infusion of normal rat plasma

(c) Giving amphenone (0.2 g/kg by mouth) along with the hexoestrol.

Procedures (a) and (c) bring the lipid deposition in the adrenal cortex back to normal.

When amphenone is administered (0.2 g/kg by mouth) for 2-17 days, the secretion of corticosterone per g gland is at the upper reaches of or above normal; secretion per gland is greatly enhanced. Large doses infused into the femoral vein inhibit secretion. The site of action of amphenone is discussed.

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THE CARBON DIOXIDE CONCENTRATION GRADIENT BETWEEN THE FETAL AND MATERNAL BLOODS OF SHEEP AND GOATS†

The observations to be presented here were made in an effort to gain a wider understanding of the circumstances that influence or modify the exchange of the respiratory gases between the fetal and maternal bloods in the placenta through a study of the exchange of carbon dioxide between fetus and mother across the five-layered syndesmochorial placenta of the sheep and goat. We sought (i) to determine the concentrations of the physically dissolved CO_2 of the plasma bicarbonate and of hydrogen ions that exist in the fetal and maternal bloods in the steady state and (ii) to gain some knowledge of the manner in which these concentrations in fetal plasma respond to variations in the individual levels of the corresponding constituents of the maternal plasma. Our hopes were not fully realized, for a steady state across the placenta is not easily maintained in circumstances that permit the fetal blood to be sampled; nevertheless, the data we have obtained do permit some inferences to be drawn about the steady state equilibrium as well as the manner in which a fall in the dissolved CO_2 and bicarbonate of the maternal plasma affects the fetal acid-base balance.

MATERIALS AND METHODS

The individual sheep and goats‡ selected for this study were brought into the laboratory four or five days before the experiment and kept off feed for the 48 hours preceding it. In preparation for the necessary laparotomy the animals were given either 1.5 to 2 cc. of Spinocain subdurally or intravenous isotonic pentothal sodium

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‡ The sheep and goats on which these observations were made were good grade animals. The sheep, Shropshires and Hampshires, were kept as a part of their flock by Dr. and Mrs. Loeb on their farm in Bethany, just outside of New Haven. There they had the run of a good pasture with a ration of hay and grain in the winter months. The goats, kept under similar conditions, were purchased from Mrs. Rudolph Gode of Cheshire. The Loebs and Mrs. Gode provided us with the breeding dates of the ewes and does on which the estimations of fetal age are based; without their interest and co-operation our study would not have been possible.

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(*c.* 0.5 cc. per kilo of body weight of a solution made by dissolving one gram in 30 cc. distilled water). The animal was then quickly placed on its back or side and the uterus exposed through an incision that separated all of the abdominal layers at once. A small vein draining a cotyledon of the central portion of the uterine horn containing the fetus was selected and from it, without dissection or handling, a 2 to 5 cc. sample of blood was drawn under oil into a syringe whose walls had been moistened with a neutral heparin-fluoride solution to prevent clotting and glycolysis. After this sample had been drawn, the uterus was opened by a single incision at right angles to the long axis of the horn and the umbilical cord delivered with a minimum of manipulation and handling. Samples of blood—2 to 5 cc.—were then similarly drawn from the umbilical vein, the umbilical artery, and the uterine artery in that order. They were drawn in rapid succession and with the minimum of disturbance to the uterus and the fetus.

In the earlier experiments the syringes were capped and put into a beaker containing melting ice and water. Following the removal of samples for the determination of the oxygen content and capacity, the syringes were transferred to a shallow mercury bath until their contents came to room temperature. The pH of the whole blood was determined by means of a glass electrode and a Beckman meter standardized with a buffer of known hydrogen ion concentration. The results were corrected to 38° C. by means of Rosenthal's[10] equation:

$$\text{Blood } pH_{38} = pH_t - 0.0147\ (38—t) \qquad (1)$$

where t is the temperature at which the pH of the blood is read. We determined by experiment that this correction factor is applicable to the bloods of sheep and goats.

The remainder of the sample was transferred under heavy paraffin oil into a centrifuge tube closed with a one-hole rubber stopper that was in turn closed with a glass rod after all but a thin layer of the oil had been displaced from the tube by the blood. The cells were separated from the plasma by centrifugation at room temperature, i.e., the temperature at which the pH was measured.

In later experiments a portion of the blood, immediately it was drawn, was transferred under oil into centrifuge tubes that had been suspended in a bath at 38° C. The separation of the cells and plasma, as well as the subsequent measurement of the plasma pH, was carried out at 38° C.; the centrifuge and the pH meter were enclosed in cabinets whose air temperature was held at that level thermostatically. The results of the two procedures were similar. The carbon dioxide content of the plasma was determined, in all cases, by Van Slyke's manometric method on 0.5 cc. samples.

The pH and the total CO_2 as determined were used to calculate the concentration of the physically dissolved CO_2 of the plasma by means of the Henderson-Hasselbalch equation:

$$pH = 6.10 + \log \frac{\text{Total } CO_2 - \text{Physically dissolved } CO_2}{\text{Physically dissolved } CO_2} \qquad (2)$$

The solubility coefficient of CO_2 in plasma is 0.0301 millimols per liter for each millimeter Hg of CO_2 pressure.[12] Accordingly, the partial pressure of carbon dioxide with which the physically dissolved CO_2 would be in equilibrium may be obtained by dividing the concentration of the latter by 0.0301. In the adult animal, where the exchange of the respiratory gases in the lungs takes place between a liquid and a gaseous phase, knowledge of the partial pressure of the gases with which the blood is in equilibrium is of obvious importance and the concentrations of the physically dissolved CO_2 are

usually expressed as gas pressures. As the exchange of the respiratory gases across the placenta takes place between two aqueous phases the movement of the physically dissolved CO_2 from the fetal to the maternal blood depends upon the gradient of its concentration across the barrier. For this reason we have expressed the concentration of the physically held CO_2 as such, though in earlier publications we referred to the pressures rather than concentrations.

RESULTS

In all 15 sheep and 3 goats were sacrificed in these experiments; their fetuses ranged from 50 to 144 days of age as reckoned from the date of insemination. Two ewes bore twins. The data obtained by the analysis of the blood samples are arranged in Table 1. The difference in the concentration of the physically dissolved CO_2 on the two sides of the placental barrier has been estimated by subtracting the value for the concentration in the uterine artery from that of the umbilical vein and correspondingly the concentration in the uterine vein from that in the umbilical artery, for within the placenta of the sheep[1]—and apparently in the goat as well (unpublished)—the fetal and maternal bloods flow in opposite directions in more or less parallel cords of capillaries. As a consequence of this arrangement the maternal blood leaving the placenta via the uterine vein is opposed to the fetal blood entering via the umbilical artery, whereas the fetal blood leaving by the umbilical vein is opposed to maternal blood entering through the uterine artery.

In every case the concentration of the physically dissolved CO_2 was higher in the fetal plasma than it was in the plasma of the opposing maternal vessel, by amounts ranging from 0.1 to 0.8 millimols per liter. The more commonly occurring difference was of the order of 0.2 millimols per liter with the tendency for the higher values to occur in the young fetuses. However, the number of observations is too limited to be conclusive in this respect.

The bicarbonate content of the fetal plasma is higher than that of the maternal in all cases but one—sheep 144 days, the last entry in Table 1—and in that case the difference is less than a millimol per liter of plasma. In the most extreme case the fetal bicarbonate concentration was 7-9 millimols per liter higher than that of the maternal plasma. The fetal plasma pH was lower than the maternal in the majority of the cases.

DISCUSSION

The data presented in Table 1 indicate that, despite wide variations in the differences in the bicarbonate and hydrogen ion concentrations of the fetal and maternal plasmas, the difference in the concentrations of physically dis-

TABLE 1

Animal	*Fetal age Days*	*Vessel*	*pH*	*$BHCO_3$ mM/l*	*Physically dissolved CO_2 mM/l*	*Difference in physically dissolved CO_2*	
						Umb.V.-Ut.A.	*Umb.A.-Ut.V.*
Sheep	50	Umb. A.	7.16			0.2	
		Umb. V.	7.43	24.4	1.1		
		Ut. A.	7.46	21.3	0.9		
		Ut. V.	7.41	21.8	1.1		
Sheep	56	Umb. A.	7.17	23.5	2.0	0.4	0.8
		Umb. V.	7.33	23.4	1.4		
		Ut.A.	7.35	17.2	1.0		
		Ut. V.	7.30	18.5	1.2		
Sheep	65	Umb. A.	7.28	24.1	1.6		0.5
		Umb. V.	...	...	...		
		Ut. A.	7.27	13.3	0.9		
		Ut. V.	7.22	14.2	1.1		
Sheep	81	Umb. A.	7.36	20.8	1.1	0.4	0.3
		Umb. V.	7.38	20.1	1.1		
		Ut. A.	7.40	13.7	0.7		
		Ut. V.	7.37	14.1	0.8		
Sheep	92	Umb. A.	7.32	23.8	1.4		0.5
		Umb. V.	7.34	21.3	1.2		
		Ut. A.	...	...	...		
		Ut. V.	7.34	15.2	0.9		
Sheep	120	Umb. A.	7.37	25.7	1.4	0.4	0.3
		Umb. V.	7.40	23.9	1.2		
		Ut. A.	7.44	17.8	0.8		
		Ut. V.	7.38	20.4	1.1		
Sheep	126	Umb. A.	7.27	22.4	1.5	0.35	0.35
	1st twin	Umb. V.	7.31	21.5	1.3		
		Ut. A.	7.40	19.0	0.9		
		Ut. V.	7.33	19.6	1.2		
	2nd twin	Umb. A.	7.21	21.3	1.7	0.3	0.2
		Umb. V.	7.27	20.0	1.3		
		Ut. A.	7.38	19.8	1.0		
		Ut. A.	7.25	20.8	1.5		

TABLE 1 (continued)

Animal	Fetal age Days	Vessel	pH	$BHCO_3$ mM/l	Physically dissolved CO_2 mM/l	Difference in physically dissolved CO_2 Umb.V.-Ut.A.	Umb.A.-Ut.V.
Goat	133	Umb. A.	7.37	31.6	1.7	0.3	0.3
		Umb. V.	7.39	29.3	1.5		
		Ut. A.	7.41	23.6	1.2		
		Ut. V.	7.35	24.9	1.4		
Sheep	136	Umb. A.	7.30	16.5	1.0	0.2	0.2
		Umb. V.	7.34	15.5	0.9		
		Ut. A.	7.43	15.2	0.7		
		Ut. V.	7.38	15.4	0.8		
Sheep	136	Umb. A.	7.35	17.0	0.95	0.1	0.15
		Umb. V.	7.42	16.4	0.8		
		Ut.A.	7.43	14.4	0.7		
		Ut. A.	7.39	15.5	0.8		
Sheep	137	Umb. A.	7.44	20.3	0.9	0.2	0.2
	1st twin	Umb. V.	7.46	19.1	0.8		
		Ut. A.	7.39	11.2	0.6		
		Ut. V.	7.36	12.7	0.7		
	2nd twin	Umb. A.	7.39	20.6	1.05	0.3	0.25
		Umb. V.	7.42	18.0	0.9		
		Ut. A.	7.39	11.7	0.6		
		Ut. V.	7.32	14.0	0.8		
Goat	137	Umb. A.	7.32	30.2	1.8	0.2	0.2
		Umb. V.	7.37	29.2	1.55		
		Ut. A.	7.42	28.1	1.35		
		Ut. V.	7.37	29.8	1.6		
Sheep	137	Umb. A.	7.47	29.3	1.25	0.2	0.25
		Umb. V.	7.49	27.7	1.1		
		Ut. A.	7.45	20.8	0.9		
		Ut. V.	7.43	22.3	1.0		
Sheep	138	Umb. A.	7.32	21.4	1.3	0.25	0.3
		Umb. V.	7.38	19.4	1.0		
		Ut. A.	7.39	14.4	0.75		
		Ut. V.	7.34	17.4	1.0		

TABLE 1 (continued)

Animal	*Fetal age Days*	*Vessel*	*pH*	*$BHCO_3$ mM/l*	*Physically dissolved CO_2 mM/l*	*Difference in physically dissolved CO_2 Umb.V.-Ut.A.*	*Difference in physically dissolved CO_2 Umb.A.-Ut.V.*
Goat	140	Umb. A.	7.33	25.8	1.5		
		Umb. V.	7.38	24.3	1.3	0.5	0.2
		Ut. A.	7.44	18.4	0.8		
		Ut. V.	7.32	21.9	1.3		
Sheep	142	Umb. A.	7.30	23.7	1.5	0.1	0.2
		Umb. V.	7.35	22.0	1.2		
		Ut. A.	7.38	20.9	1.1		
		Ut. V.	7.35	23.0	1.3		
Sheep	144	Umb. A.	7.36	26.9	1.5	0.1	
		Umb. V.	7.40	26.4	1.3		
		Ut. A.	7.45	21.0	1.2		
		Ut. V.					
Sheep	144	Umb. A.	7.39	25.9	1.3	0.15	0.1
		Umb. V.	7.43	24.5	1.15		
		Ut. A.	7.51	25.1	1.0		
		Ut. V.	7.45	26.6	1.2		

solved CO_2 on the two sides of the placental barrier is fairly constant if we consider only those fetuses 120 days or older; the average difference in concentration at the arterial ends (Umb.V.-Ut.A.) of the parallel capillary cords in this group (120-140 days) is 0.25 millimols per liter of plasma. The average difference at the venous end (Umb.A.-Ut.V.) is 0.23 millimols. In other words, it appears one may take the value 0.24 millimols per liter as a representative figure for the difference in the concentration of the physically dissolved CO_2 along the capillaries for fetuses of this group.

Results of this same order have been reported in the few cases in which similar studies were made in the cow and the goat, both animals with a syndesmochorial placenta. For example, Huggett,[5] the pioneer in the study of the placental exchange of the respiratory gases, found the carbon dioxide tension in the umbilical artery to be 18 mm. higher than that of the uterine vein, a pressure difference that corresponds to a difference in the concentration of physically dissolved CO_2 of 0.54 millimols per liter of plasma.

Keys in a study also made on goats, and in which he used a modification of Krogh's bubble method, found the CO_2 tension in the umbilical vein to exceed that of the uterine vein by 2 and 6 mm. in two cases corresponding to differences in the concentration of the physically dissolved CO_2 of *circa* 0.06 and 0.18 millimols per liter of plasma respectively. These observations were made before the pattern of counterflow in the placenta had been widely recognized, so the data are of interest though of somewhat limited significance. Keys[6] estimated the CO_2 pressures in the fetal and maternal bloods of goats in four other cases but unfortunately for our purpose the particular vessel from which the samples were drawn is not mentioned. More useful for comparative purposes are the data of Roos and Romijin[9] on the cow; this animal like the sheep and goat has a five-layered syndesmochorial placenta. Roos and Romijin estimated the difference in CO_2 pressure between the fetal and maternal bloods by referring the CO_2 content of the plasmas of samples drawn from each of the four placental vessels to the appropriate carbon dioxide dissociation curve prepared with larger samples of the fetal and maternal bloods. The four cows they studied were in the final third of the gestation period; the difference in CO_2 pressures at the arterial end of the placenta (Umb.V.-Ut.A.) they found to range between 3.5 and 32 mm. Hg and at the venous end (Umb.A.-Ut.V.) between 4 and 23 mm. Hg. These differences in pressure correspond to differences in the concentration of physically dissolved carbon dioxide of 0.1-1.0 and 0.1-0.7 millimols per liter at the arterial and venous ends, respectively, of the placental circulation.

In all of the cases described above, as well as in our own, the assumption was made that the concentrations of physically dissolved carbon dioxide in the four major vessels, uterine artery, uterine vein, umbilical artery, and umbilical vein represent the concentrations that exist at the corresponding ends of the capillaries to and from which they lead; it was further assumed that all of the blood flowing in these major vessels passes through capillaries involved in the exchange of the respiratory gases. However, the possibility must be considered that the difference in the concentrations of CO_2 between the bloods of the larger vessels may be due to the fact that the umbilical and uterine veins collect some blood that was not involved in the exchange of the respiratory gases across the placental barrier. One might assume, for example, that the concentrations of the physically dissolved carbon dioxide come into equilibrium at equality in the fraction of the fetal and maternal bloods that are involved in the respiratory exchange, but not in the remaining portion entering and leaving the uterine and umbilical veins respectively, and that at equality in the physically dissolved CO_2 the concentrations of

the total CO_2 (CO_2) of the maternal and fetal bloods are approximately equal. In that circumstance the fraction (f) of the umbilical flow involved in the respiratory exchange and coming into equilibrium with the maternal arterial blood should be:

$$f = \frac{(CO_2)_{\text{Umb.A.}} - (CO_2)_{\text{Umb.V.}}}{(CO_2)_{\text{Umb.A.}} - (CO_2)_{\text{Ut.A.}}} \quad (3)$$

If the difference between the physically dissolved CO_2 in the umbilical vein (Umb.V.) and uterine artery (Ut.A.) is 0.24 mM, the difference in total CO_2 between the two vessels should be about 1.5 mM in the steady state, as determined by reference to carbon dioxide dissociation curves of fetal and maternal bloods, i.e.:

$$(CO_2)_{\text{Ut.A.}} = (CO_2)_{\text{Umb.V.}} - 1.5 \quad (4)$$

Assuming an A-V difference of about 1.5 mM as representative of normal conditions of uterine blood flow:

$$(CO_2)_{\text{Umb.A.}} = (CO_2)_{\text{Umb.V.}} + 1.5 \quad (5)$$

In these circumstances equation 3 becomes:

$$f = \frac{(CO_2)_{\text{Umb.V.}} + 1.5 - (CO_2)_{\text{Umb.V.}}}{(CO_2)_{\text{Umb.V.}} + 1.5 - (CO_2)_{\text{Umb.V.}} + 1.5} = 0.5 \quad (6)$$

Thus, to account for our results on the basis of the above assumption would require that more than 50 per cent of the uterine blood flow be shunted through channels not involved in the exchange of the respiratory gases. Such a possibility appears to be excluded by inspection, for the organization of the vascular bed of the uterus does not appear to include a by-pass of that magnitude.[1]

On the basis of the above considerations we have accepted 0.24 millimols per liter as representative of the difference in the concentration of the physically dissolved CO_2 in the plasmas of the maternal and fetal placental capillaries of the sheep during the last month of the gestation period and have used this representative value in an effort to learn something about the physical characteristics of the placental barrier as well as the concentrations of the bicarbonate and hydrogen ion that might be representative of the normal conditions *in utero*.

The difference in concentration of the physically dissolved CO_2 on the two sides of the placenta provoked the question: in regard to the restriction it places upon the diffusion of carbon dioxide and oxygen does the placenta act as though it were a simple layer of water separating the two plasmas?

On that assumption, i.e., that the placental barrier may be regarded as a layer of water, the quantity of gas moving from the fetal to the maternal plasma, or vice versa, may be expressed according to Fick's law, as follows:

$$Q = \frac{-k\,A\,\Delta\,C}{D} \tag{7}$$

where Q equals the quantity of gas in millimols transferred per unit time, k the diffusion constant, A the area across which diffusion takes place, D, the thickness of the separating membrane, and Δ C, the difference in concentration across the membrane. The R.Q. of the sheep fetus at term is about one (unpublished data). Accordingly, the quantity of oxygen crossing the placental barrier per unit time is equal to that of the carbon dioxide moving in the opposite direction and it follows that:

$$Q_{O_2} = Q_{CO_2} \tag{8}$$

and further that

$$\frac{k_{O_2} A \Delta C_{O_2}}{D} = \frac{k_{CO_2} A \Delta C_{CO_2}}{D} \tag{9}$$

when k_{O_2} and k_{CO_2} represent the diffusion constants of oxygen and carbon dioxide respectively, and ΔC_{O_2} and ΔC_{CO_2} the difference in concentration of O_2 and CO_2, respectively, across the placental barrier. Since A, area and D, distance—the effective dimensions of that barrier—are common to both sides of the equation, it may be reduced to:

$$k_{O_2} \Delta C_{O_2} = k_{CO_2} \Delta C_{CO_2} \tag{10}$$

or

$$\frac{k_{O_2}}{k_{CO_2}} = \frac{\Delta C_{CO_2}}{\Delta C_{O_2}} \tag{11}$$

The diffusion coefficients for carbon dioxide and oxygen in water are, respectively, —1.378 and —1.607 millimols per square centimeter per day.[11] Substituting these values in equation 8

$$\frac{1.607}{1.378} = \frac{\Delta C_{CO_2}}{\Delta C_{O_2}} \tag{12}$$

and

$$\frac{1.607 \Delta C_{O_2}}{1.378} = \Delta C_{CO_2} \tag{13}$$

According to our earlier studies[8] the difference in oxygen pressure in the maternal and fetal plasmas of the gravid sheep is about 40 mm. Hg through the last one hundred days of gestation. This pressure difference is equivalent

to a difference in concentration of the dissolved gas of 0.053 millimols per liter of plasma.

(Solubility constant of $O_2 \times$ oxygen pressure in mm. Hg or 0.00132×40).

$$\text{Substituting:} \qquad \frac{1.607 \times 0.053}{1.375} = 0.061 \tag{14}$$

This, 0.061 millimols, is a difference in concentration that is about one-fourth of our observed value. The inference is that with respect to the exchange of the respiratory gases the tissue barrier of the placenta cannot be regarded as a simple water layer. At the moment no explanation of the discrepancy between the observed and the calculated values is at hand, but the possibility that the solubilities of oxygen and carbon dioxide are relatively different in the placental barrier than they are in water should not be overlooked.

The second question to which we directed our attention was: are the observed differences in the concentrations of the bicarbonate and hydrogen ions of the fetal and maternal plasmas in accord with the view that the placental barrier acts as a semipermeable membrane impermeable to the proteins and permeable to all the ions quantitatively more important such as Na^+, K^+, Cl^-, and HCO_3^-? If such is the case, the concentrations of these ions on the two sides of the membrane should be in accordance with a Donnan ratio.

The Donnan ratio, r, across the placenta in the sheep may be estimated from the following rata: (i) the colloidal osmotic pressures of the fetal and maternal plasmas and (ii) the mean molecular weights of the fetal and maternal plasma proteins. In our calculations the following assumptions are made: (i) that the maternal and fetal plasmas are comparable to solutions of proteins on the alkaline side of the isoelectric point and a diffusable monovalent electrolyte of a concentration of 150 millimols per kilogram of water, and (ii) that the difference between the colloidal osmotic pressure as measured and the pressure exerted by the proteins per se, as calculated from the mean molecular weight and the concentration of the plasma proteins, is due to the unequal distribution of the diffusible ions.

The difference in concentration (C_m) due to the unequal distribution of the diffusible ions between the maternal plasma and an outside solution of the same composition but free of protein is:

$$150 \left(r_m + \frac{1}{r_m} - 2\right) = C_m \tag{15}$$

where r_m stands for the Donnan ratio between the two solutions. The

corresponding concentration difference (C_f) when fetal plasma is used will be:

$$150\ (r_f + \frac{1}{r_f} - 2) = C_f \tag{16}$$

where r_f represents, as in the case of the maternal plasma, the Donnan ratio between the solutions. When the two plasmas are separated from each other by a semipermeable membrane the difference in ionic concentration on the two sides due to the Donnan effect (C) will be:

$$C_m - C_f = C \tag{17}$$

and the Donnan ratio between the two solutions (r) will be:

$$\frac{r_m}{r_f} = r \tag{18}$$

The colloidal osmotic pressures of the fetal and maternal plasmas of goats and sheep have already been reported.[8] The mean molecular weight of the proteins of the plasma of the maternal sheep has been estimated from the data of McCarthy.[7] The protein concentration of the maternal sheep plasma has been assumed to be 90 grams per kilogram of water; the mean molecular weight is 100,000 and the osmotic pressure due to the proteins alone corresponds to a concentration of 0.9 milliosmols. The colloidal osmotic pressure of the maternal plasma corresponds to a concentration of 1.45 milliosmols. Accordingly:

$$C_m = 1.45 - 0.9 = 0.55 \tag{19}$$

and

$$r_m + \frac{1}{r_m} = 2.00367 \tag{20}$$

$$r_m = 1.0625 \tag{21}$$

The mean molecular weight of the fetal plasma proteins is 65,000. Inasmuch as the concentration of the proteins of the fetal plasma increases with age, the value of C_f and therefore of C changes with advancing development. The approximate values for the protein concentration for the different ages are given in Table 2 together with corresponding C_f and r_f values.

At pH 7.4 the proteins of both the fetal and maternal plasmas are both on the alkaline side of their isoelectric points. The protein concentration is higher on the maternal side and C_m is greater than C_f. Therefore the positive ions should be at higher concentration on the maternal side and the negative ions at the higher concentration on the fetal.

TABLE 2

Fetal age	*Protein conc. gr/Kg water*	*H_2O per liter of plasma ml*	*Prot. con. g %*	*COP 38° C. mm H_2O*	*COP milliosmols*	*Osmotic pressure of proteins alone*	C_f'	r'_f	C	r
60	20	970	1.94	90.5	0.343	0.307	0.0364	1.016	0.51	1.046
80	24	965	2.32	112.0	0.425	0.368	0.0565	0.019	0.48	1.042
100	32	960	3.07	157.0	0.595	0.491	0.1044	1.027	0.45	1.035
120	43	945	4.06	227.0	0.860	0.659	0.2010	1.037	0.35	1.025
140	49	940	4.60	270.0	1.023	0.751	0.2715	1.043	0.28	1.019

Assuming, for purposes of illustration, that between 120 and 140 days' gestation age when the Donnan ratio r (see Table 2) is 1.025 — 1.019, the bicarbonate content of the maternal plasma to be 26.0 millimols per kilogram of water and its pH at 7.4, according to the above reasoning the bicarbonate concentration in the fetal plasma should be 1.025 × 26 or 26.6, i.e., 0.6 millimols higher than that of the maternal and the pH of the fetal plasma 0.01 units above the maternal.

In this connection, attention may be directed to the observation that the alkali reserve of the maternal and fetal plasmas from animals 111-140 days' gestation age was found to differ by less than one millimol in four out of five cases.[3] This observation is in agreement with the considerations set forth above, i.e., that if the maternal and fetal plasmas were exposed to the same carbon dioxide pressure, their bicarbonate and hydrogen ion concentrations should be approximately equal.

In vivo, due to the continuous production of carbon dioxide by the fetus the concentrations of the physically dissolved carbon dioxide on the two sides of the placental barrier are not allowed to reach equilibrium; the concentration as our data indicate is always about 0.24 millimols per liter higher on the fetal side. Since

$$CO_2 = \frac{1}{K_1} \cdot (HCO_3) \cdot (H) \qquad (22)$$

when (HCO_3) and (H) represent the bicarbonate and hydrogen ion concentrations respectively and K_1, the first apparent dissociation constant of carbonic acid, if the concentration of the physically dissolved CO_2 is higher on the fetal side, (HCO_3) and/or (H) must be higher on that side. Unless the properties of the system are more complex than we have assumed them to be, it is difficult to visualize a mechanism whereby the side having the higher concentration of physically dissolved CO_2 could have a lower bicarbonate and/or hydrogen ion concentration.

Assuming, therefore, that in the steady state the bicarbonate and hydrogen ion concentrations on the fetal side must be equal or higher, but not less than those on the maternal, one can calculate that for a difference in the concentration of the physically dissolved CO_2 of 0.24 millimols on the two sides of the placental barrier, the bicarbonate concentration should be from 0 to 5 millimols higher, per kilogram of water, and the pH from 0 to 0.08 units lower on the fetal side. As can be judged from a study of the data in Table 1, the results of our experiments are in good agreement with the above considerations.

In some cases the pH of the fetal blood was found to be more alkaline than that of the maternal in the corresponding vessel—comparing uterine artery with umbilical vein and uterine vein with umbilical artery—and the bicarbonate concentration has been found to be higher than expected or slightly below (sheep 144 days, Table 1) the maternal level. The results in these cases may be brought into accord with the reasoning outlined above, i.e., that the placental barrier can be considered to act as a simple semipermeable membrane with regard to the physically dissolved CO_2, bicarbonate and hydrogen ions — by assuming that the fetal and maternal plasmas were not in a steady state when sampled—that they were being altered or had been altered by some stress acting on the mother.

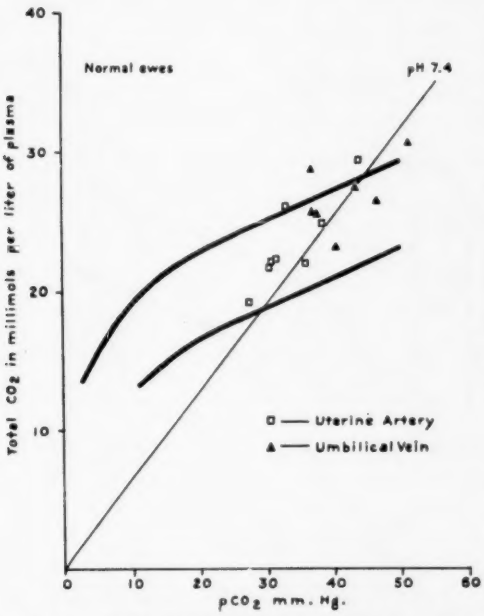

FIG. 1. Graph illustrating the relationship between the total CO_2 contents of the plasmas of normal ewes and that of their fetuses as obtained in the experiments described above. The heavy dark lines represent the limits of variation in the CO_2 content of true plasmas of normal ewes whose bloods were sampled directly from the jugular vein without anesthetic or manipulation.

We have observed for example that the anesthetic and procedures associated with the delivery of the uterus frequently result in a fall of several millimols in the total CO_2 of the maternal plasma—a drop that may occur within the half hour after the beginning of the experiment. Quite frequently the first effect on the mother results in hyperventilation.

That the bicarbonate level of the fetus follows the maternal as the bicarbonate concentration of the cerebrospinal fluid follows the concentration in the plasma,[4] with a delay imposed through the limitation to free diffusion by a tissue barrier, is suggested by a comparison of the data represented in Figures 1 and 2. In the former, the CO_2 content and pH of the maternal

arterial blood are of such magnitudes as to indicate the alkali reserve of each individual was within or above the normal range found to be characteristic for pregnant ewes.[8] In these individuals the total CO_2 of the fetal plasma tends to be slightly higher and toward the upper limit of the normal range for the mothers. When the ewes are acidotic, i.e., the total CO_2 of the maternal plasma is below the normal level—that of the fetal plasma tends to be lower as well. The delay with which the bicarbonate level of the fetus follows that of the mother is the subject of current investigation.

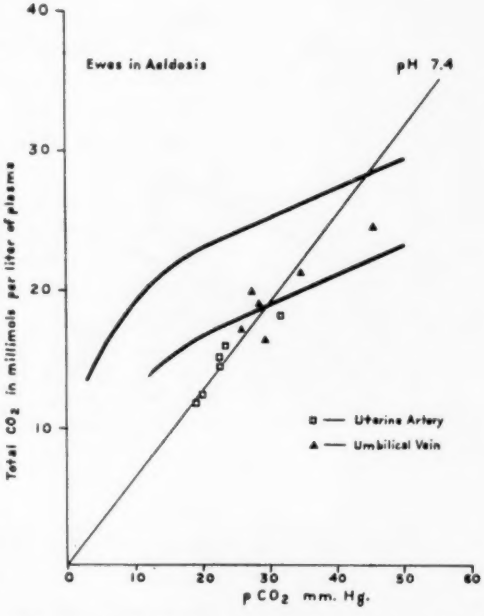

FIG. 2. As in Figure 1 except the ewes were in a state of acidosis as shown by the fact that the total CO_2 content of the maternal plasmas was in each case below the normal range.

SUMMARY

The concentrations of the dissolved CO_2, the bicarbonate and hydrogen ion in the plasma drawn from each of the four principal vessels serving the placenta of the sheep and goat—i.e., the uterine artery, uterine vein, umbilical artery, umbilical vein—have been determined at several stages during the gestation period. The data indicate that the concentration of the dissolved CO_2 on the fetal side of the placental barrier is about 0.24 millimols per liter higher during the last month of the gestation period.

During the same period the bicarbonate concentrations of the fetal plasma ranged from —0.17 to +7.9 mM/l and its pH from —0.11 to +0.08 of a unit with respect to the corresponding values in the related maternal uterine vessels. This variability in the pH and bicarbonate values of the fetal plasma with respect to the maternal is probably due to the circumstance that a sampling of the maternal and fetal bloods in a steady state condition is

rarely achieved. What the bicarbonate and pH values of the fetal plasma with respect to the maternal are in the steady state is discussed.

The concentration gradient of the dissolved CO_2 between the fetal and maternal plasma as reported here has been used as a basis for speculation about the physical properties of the placental barrier.

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PAPER ELECTROPHORETIC STUDY OF SOME COMPONENTS OF SALIVA†

Human saliva is an easily obtained, abundant secretion, but the native proteins in it seem to have been little studied. Only salivary amylase has been isolated in a pure state. In regard to electrophoresis, the phase boundary apparatus has been used by Blix[2] to study animal submaxillary mucin, by Bernfeld, Staub, and Fischer[1] and Muus[18] to test the homogeneity of purified salivary amylase, and by Mack, Wolf, and Stern[12] to investigate salivary proteins in gastric juice. Paper electrophoretic separation of protein elements in whole saliva was previously reported by one of us.[7] In this study, paper electrophoresis was used to investigate concentrated and non-concentrated saliva with and without the *in vitro* addition of bromphenol blue, calcium,[45] and iron.[50] The migration of the salivary blood group specific substances A and B was also studied.

METHOD

Paper electrophoretic apparatus used was a modification of that of Köiw, *et al.*[10, 11] Acetate, phosphate, and veronal buffers, pH 4.5-8.6, ionic strength 0.05-0.015, were used along with Munktell No. 20 filter paper. The potential ranged from 100-240 V, currents from 0.5-5.1 mA per strip, and running times varied from 1½ to 12 hours. After electrophoresis, the strips were dried for half an hour in a 70° C. oven. Some strips were subsequently stained with amido-black.[4, 8]

Autoradiography was accomplished by placing the filter paper against a strip of medical x-ray film (Kodak No-screen) and arranging the two so as to receive even pressure and contact during exposure. Identification marks were cut into the edges of both strips in order to permit reconstruction of their relative positions after completion of autoradiography. Films were developed three minutes, fixed twice the clearing time, and washed in running water for one hour.

The effect of saliva on the isoagglutinin titer was determined immediately after electrophoresis by cutting the wet filter papers into 0.5 cm. strips, placing these strips

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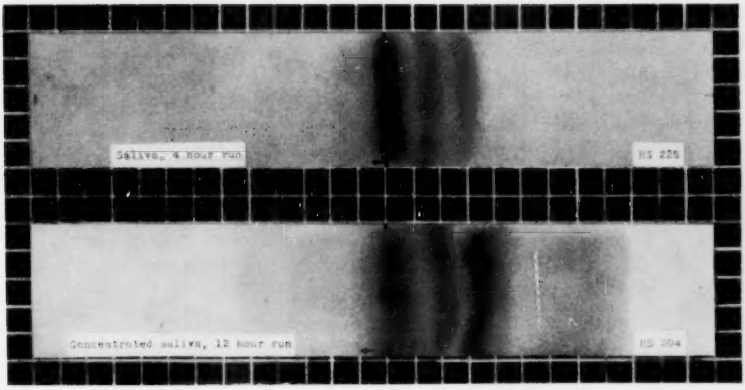

FIG. 1. Photographs of electrophoretic patterns of human saliva.

A. (upper). Unconcentrated saliva, 4-hour run. B. (lower). Concentrated (10 times) saliva, 12-hour run. Other electrophoretic conditions were: 0.1 ml sample, 100 V, 1.8 mA, KH_2PO_4-NaOH buffer, pH 6.2, μ 0.015, Munktell No. 20 filter paper. Starting points are indicated near the arrows which point toward the anode. Background is a centimeter grid.

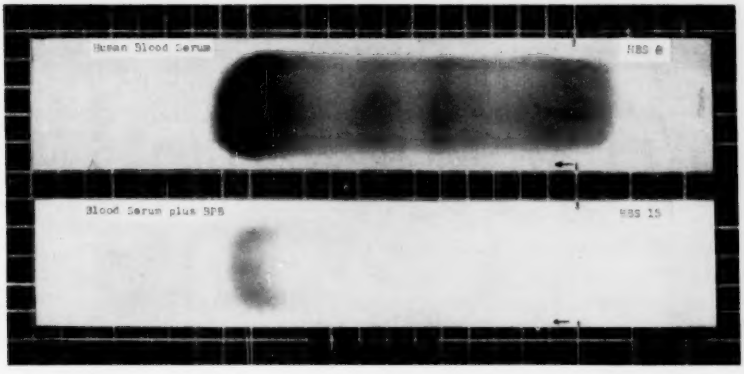

FIG. 2. Photographs of electrophoretic patterns of human blood serum.

A. (upper). Pattern was stained after electrophoresis with amido-black. B. (lower). Bromphenol blue (BPB) was added to the serum before electrophoresis. Note diffuse zone of stain ahead of the albumin fraction. Runs were 6 hours, 0.06 ml serum, 200 V, 3.0 mA, veronal buffer, pH 8.6, μ 0.05, Munktell No. 20 filter paper. Starting points are indicated near the arrows which point toward the anode. Background is a centimeter grid.

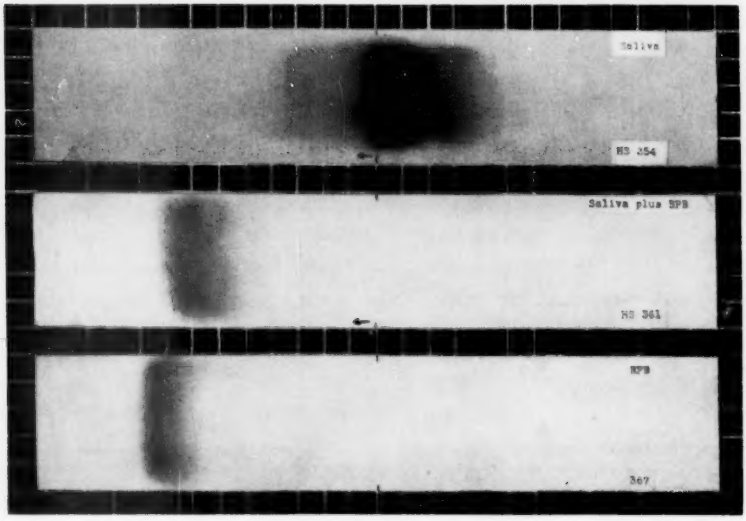

FIG. 3. Photographs of patterns of human saliva plus bromphenol blue (BPB). A. (upper). Pattern was stained after electrophoresis with amido-black. B. (middle). Bromphenol blue was added to the saliva before electrophoresis. C. (lower). Buffer plus bromphenol blue. Note decreased migration and increased "trailing" of saliva plus BPB compared to buffer plus BPB. Runs were 6 hours, 0.1 ml 10 times concentrated saliva (other conditions as in Fig. 1). Starting points are indicated near the arrows which point toward the anode. Background is a centimeter grid.

in test tubes and eluting the salivary fractions with 1 ml. anti-sera diluted 1 : 80 with 0.9 per cent NaCl. After mechanical shaking for 10 minutes, tube agglutination tests were carried out with 0.2 ml. of the fraction dilution, progressively doubled, and 0.1 ml. of a 2 per cent suspension of washed red cells. Drops of each mixture were read with use of a microscope under low power after all samples had been allowed to stand at room temperatures for one to two hours.

EXPERIMENTS AND RESULTS

Unconcentrated saliva. Saliva samples were collected on ice from seven healthy subjects. Each sample was centrifuged in a cold room (4° C.) and some of the supernatant fluid was used immediately for electrophoresis.

Figure 1A shows an amido-black stained pattern under conditions resulting in the clearest separation. On the cathode side two distinct fractions can be seen, plus a zone near to, and indistinct from, the material which remained at the starting point (i.e., the place where the sample was applied). On the anode side a diffuse fraction, manifested usually by a trailing, was evident.

Reproducible patterns resulted with saliva from only two of the donors; the others apparently did not always have sufficient organic matter in their saliva to produce a distinct pattern.

Concentrated saliva. Some samples of the above were frozen and dried *in vacuo*. The residue was re-dissolved in enough of one of the following solvents to effect a concentration ranging from 3 to 10 times: (i) distilled water, (ii) glycerol and distilled water, (iii) same buffers as were used for electrophoresis. Clearest separation were obtained with samples dissolved in diluted glycerol.

Figure 1B shows a pattern similar to Figure 1A except for time and sample. Two sharp fractions and two diffuse fractions can be seen on the cathode side, whereas the anode side displays two fraint fractions, one appearing mainly as a trailing. Some material remained at the starting point.

Using 10 times concentrated saliva dissolved in one part glycerol to three parts distilled water as a sample and a KH_2PO_4– NaOH mixture, pH 6.2, μ 0.015, as a buffer, consistent and clear results could be obtained with all salivas tested. Therefore, these conditions were used in all subsequent experiments involving concentrated saliva.

Saliva plus bromphenol blue. If one adds a very small amount of bromphenol blue (BPB) to blood serum, only the albumin absorbs the dye,[8, 11] permitting one to note the location of the albumin after electrophoresis (Fig. 2). But, in all of our serum plus BPB patterns, a diffuse zone of stain appeared in advance of the distinct albumin-BPB zone (Fig. 2B). This

diffuse extension of stain seems neither due to protein migration, as it was not visible in the amido-black stained pattern (Fig. 2A), nor due to BPB alone (cf. below). It may be a manifestation of an inhomogeneity of the albumin-dye complex.

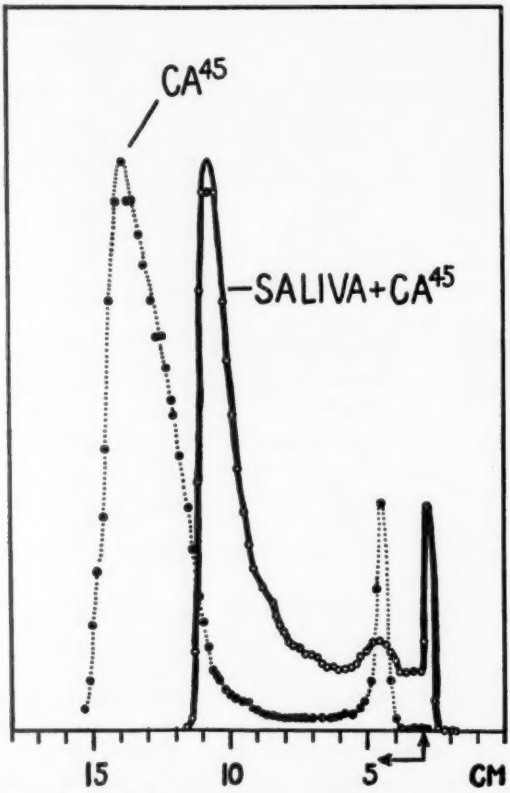

FIG. 4. Radioactivity distribution along an electrophoretic pattern of saliva plus $Ca^{45}Cl_2$ compared to that of a pattern of buffer plus $Ca^{45}Cl_2$. Arrows indicate starting point and direction of migration toward the anode. Radioactivity was measured photometrically from autoradiographs of patterns. Runs were 6 hours, 0.06 ml 10 times concentrated saliva (other conditions as in Fig. 1).

The BPB-binding ability of saliva was investigated by adding 0.1 ml of a 1 per cent aqueous BPB (Harleco) solution to 0.4 ml of 10 times concentrated saliva. After one hour, samples were used for electrophoresis. As a control, similar samples of BPB diluted with buffer were used, one saliva

and one control sample being arranged alongside each other between the same pair of buffer-containing cells and the starting points aligned so as to be equidistant from the cells.

Figure 3 shows patterns as revealed by pre-electrophoresis addition of BPB as compared to post-electrophoresis staining with amido-black. The pattern of the saliva plus BPB appears as a single zone toward the anode. A similar 6-hour migration pattern, as assessed by amido-black staining, shows beginning zone separation on the cathode side, but no anode zone corresponding to that of saliva plus BPB. Buffer plus BPB migrates anodically as single zone, but with two consistent differences compared to saliva plus BPB, always farther and with a more narrow zone.

Saliva plus Ca^{45}. The addition of small amounts of a $Ca^{45}Cl_2$ solution to saliva was carried out to determine if this procedure resulted in any *in vitro* binding by salivary fractions. With a micropipette, 10 λ and 20 λ of a solution of $Ca^{45}Cl_2$ (specific activity about 300 mc/gm) were added to 1 ml samples of concentrated saliva. After one hour, 0.1 ml samples were used for electrophoresis. Also used were similar amounts of the $Ca^{45}Cl_2$ solution added to the buffer. After electrophoresis, autoradiographs were made before and after staining.

Figure 4 reveals the results after using a photometric device* to scan the autoradiographs. Radioactivity was detectable toward the anode, but the following differences were noted: (i) saliva plus Ca^{45} curve displayed a peak at the starting point, (ii) both curves showed peaks in an identical location on the anode side near the starting point, but the saliva peak was always lower and less sharp, (iii) both curves had maximum peaks of radioactivity toward the anode, but the Ca^{45} peak was always distinctly ahead of the saliva, (iv) radioactivity between the peaks was much greater in the saliva curve, particularly there was an increasing amount of radioactivity from the small peak to the large peak.

Samples containing saliva plus both Ca^{45} and bromphenol blue indicated that the location, i.e., the distance from starting point, of the BPB (Fig. 3B) corresponded almost exactly with the location of the maximum peak of radioactivity.

When patterns were stained with amido-black (Fig. 3A), no distinct anodic zones were visible which could coincide with the localization of peaks of saliva plus Ca^{45} as indicated by radioactivity.

Saliva plus Fe^{59}. $Fe^{59}Cl_3$ was added to saliva in order to observe any binding action or change in mobility of the salivary fractions. To 0.1 ml of

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concentrated saliva was added 160 γ-per cent and 320 γ-per cent of an $Fe^{59}Cl_3$ solution (specific activity about 600 mc/gm). Applied to the paper were 0.05 and 0.1 ml samples. After electrophoresis, autoradiographs were made.

No differences could be noted when stained patterns of saliva and saliva plus Fe^{59} were compared. Autoradiographs revealed radioactivity only at the point of sample application. This occurred in 34 runs using various combinations of electrophoretic conditions and different saliva samples.

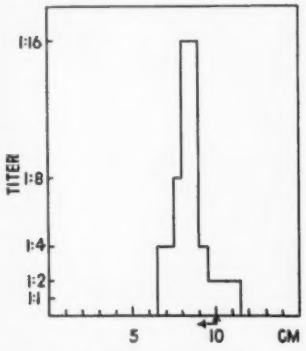

FIG. 5. Blood group specific substance A titer in an electrophoretic pattern of human saliva. Arrows indicate starting point and direction of migration toward the anode. Run was 10 hours, 0.1 ml 3 times concentrated saliva, 100 V, 1.5 mA, veronal buffer, pH 8.6, μ 0.05, Munktell No. 20 filter paper.

Blood group specific substances in saliva. The presence of blood group specific substances in saliva is well established.[14] Salivas of certain individuals (secretors) contain these substances in much higher concentration than other individuals (nonsecretors) of the same blood group.[14] With use of salivary inhibition of agglutination as a method, it appeared possible to locate the active substances in the filter paper following electrophoresis.

Saliva from secretors was concentrated 3-10 times and samples were applied to the filter paper. After electrophoresis, in all of the 19 runs with Group A saliva, an inhibition of agglutination could be noted extending from the place of sample application toward the anode (Fig. 5). Of the various buffers tried, the use of veronal resulted in the clearest migration. Using Group B saliva, the only inhibition apparent was around the starting point.

DISCUSSION

Because the composition of saliva is not constant,[15] attempts to develop diagnostic "levels" of salivary constituents have not been too successful. Even though the secretory products from salivary glands may change in concentration, the relative amount of each element secreted by each kind of cell, or group of cells, may not vary. Each gland cell may produce the same proportion of elements at any time, and, in unit times, the total amount secreted may vary, but the proportion may be unaffected.

In all of our electrophoretic studies on saliva, we have noted a fairly constant pattern of protein separation as assessed by stain location and intensity on the paper from electrophoretic runs under similar conditions. This has been interpreted as evidence that the proportion of salivary fractions is also fairly constant. In addition, blood group specific substances (Fig. 5) indicate fairly reproducible migration characteristics under constant conditions.

Saliva usually contains insufficient amounts of protein for electrophoretic purposes, but the salivas of some individuals seem concentrated enough for immediate use (Fig. 1A). Convenient concentrations can be obtained by dehydrating saliva, but some of the necessary techniques may result in the native proteins becoming denatured. Our use of low temperatures during the preparative procedures largely prevented denaturation as far as could be judged by comparing the patterns with those from nonconcentrated saliva (Fig. 1).

The change in mobility of bromphenol blue after addition of saliva (Figs. 3B and 3C) may be explained by assuming that the dye combines with some component of saliva. This salivary component was not apparent on amido-black stained patterns (Fig. 3A). Although no blood color was visible in any of our saliva samples, it is possible that small amounts of serum proteins were present in the mouths of the donors. But the amount of serum proteins in saliva was not enough to be stained subsequently with amido-black (Fig. 3A).

In Figure 4 the differences in the radioactivity curves between Ca^{45} and saliva plus Ca^{45} can also be interpreted as evidence of a combining between Ca^{45} and some component of saliva. Both these differences and that above with BPB may be due to some changes in the charge of the salivary proteins and Ca^{45} or BPB or both. It is possible that the same salivary component influences the mobility of Ca^{45} and BPB because, with similar electrophoretic conditions, the peak of saliva plus BPB (Fig. 3B), as assessed by stain distribution, was in the same location as the leading peak of saliva plus Ca^{45}, as measured by radioactivity. Further experiments are necessary to clarify the problem.

Horst and Schäfer[8] reported differences in Fe^{59}-binding by serum proteins depending on the concentration of the iron. Using similar concentrations, we could find no evidence of *in vitro* Fe^{59}-binding by migrating salivary proteins.

Preliminary investigations have revealed that no differences could be detected in the stained electrophoretic patterns of saliva from persons with

and without pathological states. However, demonstration of biologically active fractions in saliva, such as enzymes,[7] antibacterial factors,[8] and blood group specific substances (Fig. 5), offers additional opportunity to determine if any differences exist. Further investigations in this regard are ni progress.

SUMMARY

Reproducible paper electrophoretic patterns of saliva were obtained.

The salivas of some individuals contain enough protein for immediate use. Patterns showed two distinct fractions toward the cathode and a diffuse trailing toward the anode.

Concentrated (by freeze-drying) saliva samples appeared not to be denatured. After electrophoresis, two distinct and two diffuse fractions appeared toward the cathode and one diffuse fraction and a trailing could be noted on the anode side.

Addition of saliva to a bromphenol blue solution and a $Ca^{45}Cl_2$ solution influenced the mobility of these two compounds. This influence was interpreted as a combining with salivary proteins. The same salivary fraction appears to bind both.

No *in vitro* $Fe^{59}Cl_3$-binding by migrating salivary proteins could be demonstrated.

Migration of blood group specific substance A in saliva was demonstrated.

Possible biological significance and clinical application of these results have been discussed.

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The Board of Editors

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DEVELOPMENT OF PHAGOCYTIC ACTIVITY IN THE RETICULO-ENDOTHELIUM OF THE ALBINO RAT: A COMPARISON OF PRENATAL, NEONATAL, JUVENILE, AND ADULT PERIODS**†

INTRODUCTION

Although there have been numerous investigations on the macrophagic or reticulo-endothelial system of adult mammals, relatively few studies have been made on the phagocytic potentialities of fetuses and young mammals. Emmel,[19] in an abstract on the hematogenic activity of the pig embryos' liver, commented on the phagocytic activity of the hepatic "endothelial" cells. Phagocytic capacity of the reticulo-endothelial system is present in the rabbit.[40] Battaglia[10] injected carmine into 5 dog fetuses through the umbilical arteries. Although carmine collected in the pulmonary arteries, phagocytosis by alveolar phagocytes was not evident; von Kupffer cells segregated carmine granules only rarely. Culbertson[15] found that rats of younger age groups phagocytosed less trypan blue than did adults.

Italian investigators have made contributions in the vital staining of living human fetuses and prematures. Dellepiane[16] injected colloidal mercuric sulphide into human fetuses of 3, 5, and 6 months gestational age and found phagocytosis in the liver and spleen beginning in the third month. Using Dellepiane's method, Nizza[39] studied human fetuses older than 6 months gestational age. He confirmed Dellepiane's findings and concluded that phagocytic activity was more marked in the liver than in the spleen. Bracco[13] compared the macrophagic potentialities of fetal placental blood of premature and term babies and concluded that the premature child has less phagocytic potentialities.

As far as is known, no one has attempted to investigate the reticulo-endothelium in its chronological development as a macrophagic system. The

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purposes of this investigation, therefore, were (i) to determine *in vivo*, as far as the method permits, the stages of fetal development in which the macrophagic system begins to function in the albino rat, i.e., when free and fixed macrophages begin to exhibit phagocytic powers, (ii) to determine the sequential development of phagocytosis in various organs and tissues, (iii) to trace the macrophagic system in its functional capacities from prenatal through postnatal periods.

TABLE 1. THE NUMBER OF EXPERIMENTAL AND CONTROL ANIMALS STUDIED AT SPECIFIC FETAL AGES WITH THE NUMBER OF DEATHS OCCURRING IN ADULTS AND FETUSES. FETAL RATS 15 DAYS OR OLDER WERE USED IN BOTH INTRA-AMNIOTIC AND INTRA-EMBRYONIC SERIES OF EXPERIMENTS

Exp. Series I: Intravenously injected pregnant rats.
Exp. Series II: Intra-amniotically injected fetuses.
Exp. Series III: Intra-embryonically injected fetuses.

Exp. series	*No. preg. rats used*	*Fetal age in days*	*No. fetuses exp./contr.*	*Deaths adults/fetuses*	*Fetuses studied exp./contr.*
I	4	11-13	?/0	aborted	0/0
I	8	14-17	67/0	3/12	18/0
I	6	19-21	50/0	3/15	19/0
II	3	11-13	15/6	1/15	4/3
II	10	15-17	24/8	2/?	6/5
II	6	19-21	11/6	0/1	6/1
III	5	14-15	15/7	3/16	9/2
III	6	16-17	25/8	1/13	21/3
III	3	18-19	20/4	0/3	20/2
III	4	20-21	23/5	0/0	23/2
Total	43		239/44	13/75	126/19

The significance of this study must be interpreted with respect to limitations in experimental method. It was pertinent to determine the earliest date at which phagocytic activity could be detected in the fetus. Thorotrast induces deleterious effects even in adult animals and it is probable that such effects would be more severe in fetuses. It is evident, therefore, that the phagocytic responses observed in this study were accomplished under unphysiological conditions. As will be pointed out later, lymphatic drainage in the rat fetus is incompletely developed and circulation of this fluid is not well established until after birth. This means that distribution of the colloid within the fetus is relatively inefficient, and lack of phagocytic reaction may often be due not to inability of cells to take up the test material but to the fact that they do not come into adequate contact with it.

MATERIALS AND METHODS

Wistar strain albino rats were divided into prenatal and postnatal groups. These groups were subdivided into six series as shown in Tables 1 and 2.

The age of fetuses was dated from the time spermatozoa were found in vaginal smears. Four female rats were caged together overnight with two males. Vaginal smears obtained the following morning were air-dried and stained with Harris'

TABLE 2. THE NUMBER OF EXPERIMENTAL AND CONTROL ANIMALS STUDIED AT DIFFERENT POSTNATAL AGES

Exp. Series IV: Intraperitoneally injected postnatal rats.
Exp. Series V: Intracardially injected newborns through 20 days postpartum.
Exp. Series VI: Intravenously injected rats older than 25 days.

Exp. series	*Postnatal age in days*	*No. animals exp./contr.*	*Animals studied exp./contr.*
IV	2	6/0	6/0
IV	3	8/1	8/1
IV	5-7	13/3	13/3
IV	9	4/1	4/1
IV	16-17	12/1	12/1
IV	20	3/1	3/1
IV	30	3/1	3/1
IV	50	3/1	3/1
IV	70	3/1	3/1
V	1-2	18/2	18/2
V	3-4	10/0	10/0
V	6-7	8/1	8/1
V	10	4/0	4/0
V	14-17	10/2	10/2
V	20	4/0	4/0
VI	30	4/0	4/0
VI	40	4/0	4/0
VI	60	3/0	3/0
VI	70	3/0	3/0
		123/15	123/15

hematoxylin and eosin Y. Those rats with spermatozoa in the smears were presumed pregnant and segregated into separate cages.

In the first experimental group, adult pregnant rats were given intravenously a total of 0.50 cm^3 of Biebrich's scarlet, colloidal gold, or Thorotrast, and were sacrificed 24 hours after injection. In the second experimental group, pregnant rats were anesthetized with nembutal (dosage: 40 mg/kg body weight) and midabdominal incisions were made. One uterine horn was gently lifted and placed in a position suitable to visualize the fetuses through transmitted light. Between 0.01 to 0.02 cm^3 of Thorotrast was injected into each amniotic cavity of that horn. As a control at least one

amniotic cavity in the other horn was injected with 0.9% saline solution. The incision was then closed with two layers of sutures and covered with collodion. The rats were sacrificed a day after the operation. Pregnant rats in the third experimental group were treated in a manner similar to the second group, except that injections were made intraperitoneally or intrapleurally, and the animals were sacrificed 8 or 24 hours after the operation.

In the postnatal series, each young rat received Thorotrast either 5 cm^3 or 10 cm^3/kg body weight in one or two injections respectively and were injected intracardially, intravenously, or intraperitoneally. Controls were injected with equal volumes of 0.9 per cent saline. The animals were sacrificed in periods of 6, 24, 48 hours, and one and two weeks after the first injection.

Pregnant rats in the first three experimental series were sacrificed with chloroform fumes. Fetuses were removed from the uterine horns and x-rayed with a pediatric x-ray machine screened with 2 mm aluminum at 50 KVP and 15 ma with four seconds exposure on Kodak Industrial type M film. Postnatal experimental rats were sacrificed in groups of two or four with chloroform fumes and subjected to x-ray. The plates were developed immediately and temporarily fixed.

The plates were read in accompaniment with the autopsies, as this method was useful in determining the localization of the thorium salt in tissues. Major reticulo-endothelial organs (e.g. liver, spleen, and nodes) as well as those in which the salt is occasionally found (e.g. lung, kidney, and thymus) were examined. Tissue samples were then removed, fixed in aqueous Bouin's fluid, and prepared by the dioxane-paraffin method. Single sections were cut at 6 μ, while serial or interrupted sections were cut at 7 μ. The former was limited to term fetuses and postnatal animals, and the latter to fetuses younger than 20 days' gestation. The slides were stained in Harris' hematoxylin and eosin Y.

Unstained sections, mounted adjacent to stained ones, offered several advantages[8]: (i) the presence or absence of the salt in the tissues could be seen, (ii) small amounts of segregated thorium salt could be observed, (iii) the distribution of the thorium salt could be readily ascertained. In brief, this method entailed lowering the condenser and cutting down the diaphragm so that the maximal refractile property of the thorium granules was exhibited. The globules of salt appeared yellowish to yellowish-green.

OBSERVATIONS AND DISCUSSION

I. EXPERIMENTAL SERIES I AND II

Preliminary experiments were carried out by injecting two electronegative colloids into pregnant rats. Biebrich's scarlet, used by Shimidzu,[34] and colloidal gold, employed by Sato,[31] have been reported to pass through the placenta. Recently, however, Haigler and Williams[20] were unable to confirm the transplacental passage of colloidal gold, and the present investigator was unable to find either colloid in the fetuses.

Thorotrast injected into pregnant rats was not visualized by x-rays either in the placentas or fetuses; the absence of thorium salt was confirmed histologically. However, segregated thorium particles were in free macrophages in the outer portion of the myometrium of the uterus. Some of

the syncytiotrophoblastic cells phagocytosed particulate matter resembling hemosiderin in all chorio-allantoic placentas, except in one pregnant rat sacrificed on the 14th day of gestation. In the one exceptional rat, hemosiderin was abundant in the maternal lacunae of the syntiotrophoblasts, but no phagocytosis was observed. Wislocki and Dempsey[41] reported that cytotrophoblastic cells in the cat placenta phagocytosed hemoglobin derivatives, but this was not confirmed in the present investigation using rats.

Thorotrast was not found in fetuses after intra-amniotic injections but was flocculated in the amniotic cavity. The particles were probably too large to be taken into fetuses through the skin, respiratory tract, gastrointestinal tract, or fetal membranes. Similar observations were reported by Windle and Bicher[36] who attempted to demonstrate intra-uterine respiration by means of intra-amniotic injections of Thorotrast. On the other hand, trypan blue was absorbed by guinea pig fetuses through the skin, and respiratory and alimentary canals.[39] The discrepancies can be explained on the basis that trypan blue readily stains dead or dying cells, whereas thorium salt is picked up in visible amounts predominately by the reticuloendothelium.

Failure of transplacental transmission and its significance. Several investigators have demonstrated x-ray visualization of the placentas of Thorotrast-treated rodents,[14, 18, 25, 28] but they were unable to detect the passages of thorium particles into fetuses. In contradistinction, Shute and Davis[35] were unable to demonstrate placental visualizations of Thorotrast-injected rabbits, and similar results were obtained in the present rat experiments.

The sizes of the two colloids are noteworthy: colloidal gold, 20 to 200 mμ[28] and colloidal thorium dioxide, i.e., Thorotrast, 3 to 10 mμ (communication from Heyden Chemical Company). In view of the fact that Sato's work[31] has remained unconfirmed, the results should be questioned. Furthermore, if particle size is important in passing through the placenta, then the smaller thorium salt particles should get through the barrier. Although thorium is an unphysiological metal, it can enter the fetal circulation in the form of thorium nitrate solution.[26] One must conclude, then, that particle size is an important factor in the passage of substances from the maternal to fetal circulation.

II. EXPERIMENTAL SERIES III-VI

The common basis for all of these experiments is the study of macrophagic activity *in vivo* after Thorotrast injections. The presence of a colloid in a localized region is ordinarily dependent upon the route of injection. For example, macrophages of the omentum and mediastinal nodes are

readily activated after intraperitoneal injections, but not after intravascular injections. Therefore, by using different routes of injection, phagocytosis can be studied in a wide variety of locations.

A. Fixed reticulo-endothelial elements

(1) Liver

At *16 days* of gestation no phagocytosis took place in the liver. The lumens of the sinusoids were irregular in size and course and did not appear to be continuously lined with von Kupffer cells of stellate form. All the lining cells appeared elongated and had flattened vesicular nuclei. The sinusoids contained large numbers of hematopoietic cells.

At *17 days* of gestation the reticulo-endothelial cells of the liver began to function as macrophages. Hemosiderin and small globules of thorium salt were occasionally phagocytosed by von Kupffer cells (Fig. 3). Single particles were adherent to or within the endothelial cytoplasm of central veins, but it was difficult to determine whether these globules were actually phagocytosed. At this stage more reticulo-endothelial cells developed and the sinusoidal lumens became narrowed. Hematopoietic cells were still numerous.

The gestational period of the albino rat is approximately 21 days, and phagocytosis was first observed during the middle of the last gestational week. When India ink was injected into chick embryos from 2 days to 14 days old, particles of ink were found in the "endothelium" of the liver and blood vessels of the area vasculosa.[22] In human fetuses, colloidal mercuric sulphide was phagocytosed by reticulo-endothelial cells of the liver from the end of the first trimester of gestation.[16, 20] Phagocytosis was initiated at a relatively earlier stage of development in human fetuses and chick embryos than in rats. This discrepancy may be partially accounted for by differences in technique. All the cited investigators injected the colloids intravenously, while Thorotrast was injected intraperitoneally in the rats. The injected material was not absorbed from the peritoneal cavity of the fetuses but underwent flocculation, a factor which might explain the lack of phagocytic activity at the 16th day and the but slight activity at the 17th day.

At *18-19 days* of gestation the liver was histologically similar to that of the 17th day. However, the macrophages contained more thorium particles.

Up to *20-21 days* of gestation, the sinusoids were irregularly arranged. At term, rudiments of the radial arrangements of sinusoids were evident but were restricted to the region around the central veins. In general, the phagocytic ability of the reticulo-endothelial cells and number and distribution of hematopoietic cells were similar to the state at 18-19 days.

However, thorium plaques (large granules of thorium[17]) within macrophages were found abundantly in several rats.

Birth-2 days postpartum. The number of activated macrophages was greater than in the late prenatal liver. Although the cells were not usually filled with thorium salt, enough was deposited so that the liver could be visualized by x-ray (Fig. 1). Concomitantly, hematopoietic activity reduced abruptly. Erythropoietic cells, predominantly normoblasts, were found in perisinusoidal islands; granulocytic cells were not aggregated as such but were found separately in sinusoids; only a few megakaryocytes were present. The decrease in hematopoietic activity paralleled the differentiation of primitive reticulum cells towards reticulo-endothelial cells.

At *3 days* rudiments of radial arrangements of the sinusoids were similar to those of term fetuses. Although quantitative methods were not attempted, a proportionately larger number of macrophages tended to be filled with the colloid. In addition, macrophages were first noted to be somewhat evenly distributed throughout the lobules (Figs. 4 and 5). Up to this period, some lobules had macrophages distributed evenly, while most others had only a haphazard distribution. Animals sacrificed 24 hours after injection had

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PLATE 1.

With the exception of Figures 1 and 2, the following figures are from unretouched photomicrographs of tissues fixed in Bouin's fixative, sectioned at 6 or 7 μ, and stained with Harris' hematoxylin and eosin. Abbreviations: A = artery; L.V. = lymph vessel; L = lymphocyte; M = Thorotrast containing macrophage; H.M. = hemosiderin-containing macrophage; T = thorium salt globules; V = vein.

1. Ventral view of a 2-day neonatal rat injected intraperitoneally with 0.03 cm^3 of Thorotrast. The liver is visualized, while the opaque areas below the liver in the region of the spleen are Thorotrast flocculations with concentrations of thorium-laden macrophages. Note that the substernal lymphatics does not contain enough Thorotrast to be visible by x-ray. x1.36
2. Ventral view of a 3-day rat injected intraperitoneally with 0.035 cm^3 of Thorotrast. The liver is visualized with flocculations of Thorotrast in the peritoneal cavity. Note that the substernal lymphatics contain Thorotrast showing the functional development of peritoneal drainage. x1.36
3. Liver of a 17-day fetus injected intra-embryonically with approximately 0.01 cm^3 of Thorotrast 8 hours before sacrifice. This shows a hemosiderin-laden macrophage adjacent to a megakaryocyte. Note the abundance of normoblasts. x500
4. Liver of a 3-day neonatal rat injected intraperitoneally with approximately 0.02 cm^3 of Thorotrast 24 hours before sacrifice. The macrophages are the dark spots rather evenly distributed in the lobules. x121
5. Same liver as Figure 4 with arrow pointing to the same macrophages as the previous figure. The macrophages are distended with thorium globules. Islands of normoblasts are seen below the central vein. x500
6. Liver of a 30-day-old rat injected intraperitoneally with 0.30 cm^3 of Thorotrast 24 hours before sacrifice. The macrophages appear as dark masses, more concentrated in the periphery of the lobules. x75
7. Spleen of a 19-day fetus injected intra-embryonically 24 hours previous to sacrifice with approximately 0.01 cm^3 of Thorotrast. Only macrophages containing segregated hemosiderin are present in the primitive red pulp. The organ shows a myeloid structure with a developing lymphocytic peri-arterial sheath. x333

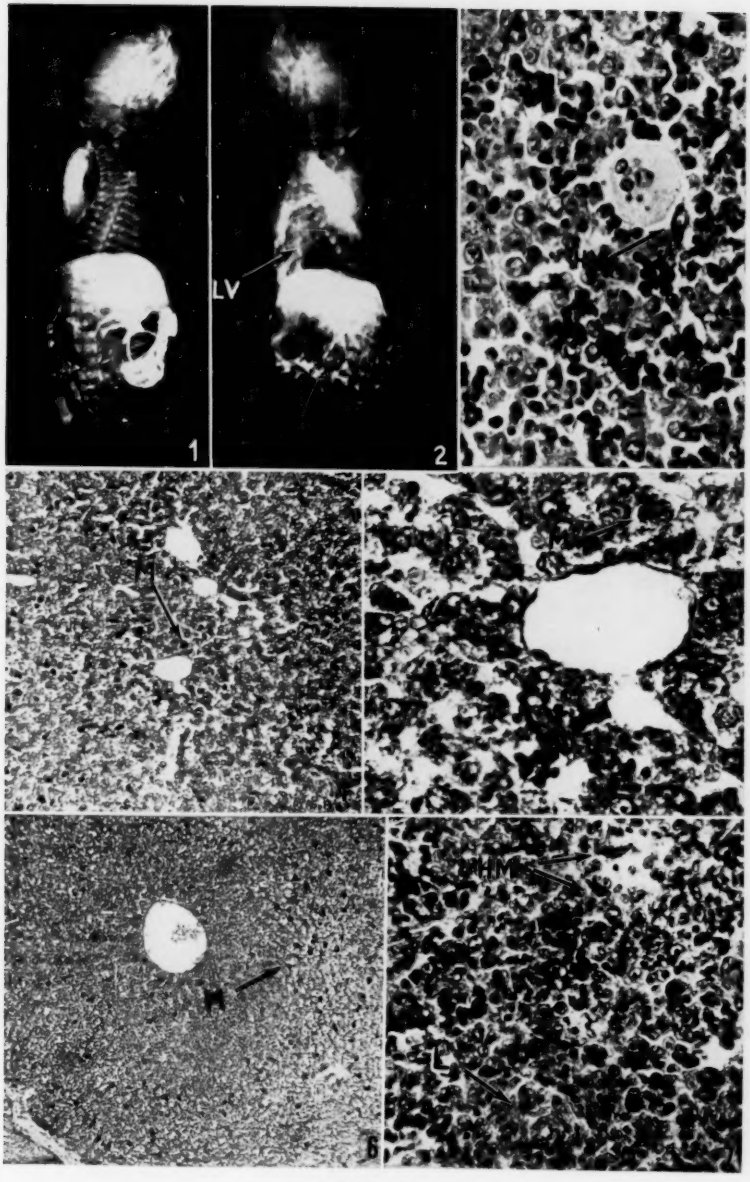

PLATE 1.

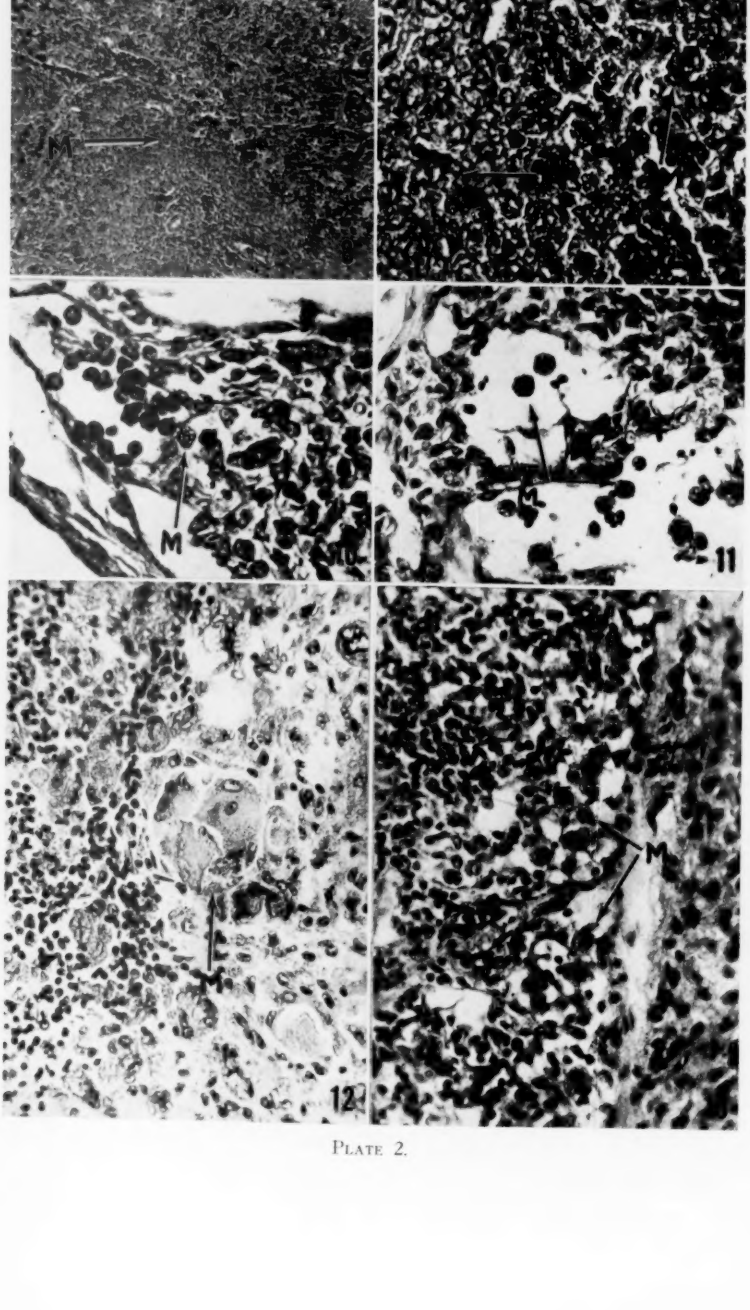

PLATE 2.

approximately the same number of activated macrophages as those killed 8 hours after injection. The macrophages in the latter series retained their stellate form, whereas, in the former, many were rounded.

At *5-7 days* some of the sinusoids extended from the central veins to the periphery of the lobules. The sinusoidal lumens became narrower by the 7th day as compared to the 3d day. Hematopoietic activity occurred only in small isolated islands, composed primarily of normoblasts. The phagocytic potentialities of von Kupffer cells were unaltered in the period between the 3d and 7th postpartal days.

At the *16th day,* most of the sinusoids reached the periphery of the lobules. The central veins were of variable sizes, even in different lobules of the same lobe. Few blood islands were found. Phagocytosis was more pronounced than in younger rats.

In three experiments, rats were injected intraperitoneally and intracardially with Thorotrast one or two weeks before sacrifice and killed when 14 to 17 days old. The macrophages were distributed evenly in the lobules, as in animals sacrificed 24 hours after injection. The livers of these rats, as compared with those sacrificed a day after injection, contained many more macrophages. Most of the phagocytes appeared round because they were loaded with plaques, whereas von Kupffer cells of those rats killed after 24 hours retained their stellate form.

At *20 days* macrophages were still evenly distributed throughout the lobules. Culbertson[15] concluded that phagocytic potentialities reached a maximum by 18 days' postpartum; no attempt was made to corroborate his findings. Blood islands were infrequent and with the cessation of hematopoiesis in the liver, the organ began to resemble the adult structure.

PLATE 2.

8. Spleen of a 20-day-old rat injected with 0.15 cm^3 of Thorotrast showing a concentration of macrophages (dark masses) in the red pulp. The Malpighian bodies are well developed. x75.

9. Spleen from same animal as Figure 8 showing the border of a Malpighian body without any macrophages to the left, and macrophages concentrated in the red pulp to the right. Normoblasts are still present. x575

10. Mediastinal node from a 21-day fetus injected intra-embryonically with approximately 0.01 cm^3 of Thorotrast. This shows a primitive macrophage with ingested particles of Thorotrast. Compare its size with adjacent red blood cells. x575

11. Mediastinal node of a 3-day-old rat injected intraperitoneally with 0.30 cm^3 of Thorotrast. The free and fixed macrophages are found predominantly in the subcapsular lymphatic sinusoids. x500

12. Multinucleated giant cell phagocyte from a mediastinal node of a 17-day neonatal rat injected intraperitoneally 5 and 7 days before sacrifice with a total of 0.50 cm^3 of Thorotrast. x333

13. Mesenteric node of a 31-day-old rat injected intraperitoneally with 0.30 cm^3 of Thorotrast. Note the macrophages are large fixed mononuclear cells closely associated with the reticulum of the medullary sinusoids. x333

At *30-40 days* the liver showed no hematopoietic activity. In animals killed 24 and 48 hours after the last injection, macrophages were more prevalent in the peripheral portions of the lobules (Fig. 6).

At *50-70 days* the liver acquired histological characteristics of the adult. As in the previous period, more Thorotrast-containing macrophages were located at the margins of the lobules. There appeared to be less macrophages per unit area in the liver of animals of this age, in comparison to younger rats.

Comments. Hepatic phagocytosis was first observed on the 17th day of gestation when it appeared only in fixed macrophages and never to any striking degree. The same elements also collected hemosiderin but in greater quantity. The amount of segregated thorium salt increased from the 17th day of gestation to 3 days' postpartum, at which time the activated macrophages were rather evenly dispersed throughout the individual lobules.

Macrophages were distributed uniformly throughout the lobules until 20 days' postpartum. Greater numbers of reticulo-endothelial cells at the margins of the lobules were phagocytic in rats 30 days or older. McKellar,[27] however, reported that von Kupffer cells were more numerous in the periphery of the lobules by the 14th postpartal day. His study was based on the cytological differentiation of von Kupffer cells which were potentially phagocytic, whereas the present study was based on the observed macrophages. Culbertson[15] concluded that the livers of nursling rats phagocytosed less particulate matter than the livers of weaned rats. Although quantitative methods were not used in the present experiments, his observations were corroborated.

When Thorotrast is injected intraperitoneally, fixed hepatic phagocytes bunch into groups of 4-10, concentrating toward the central veins within a week after injection, and three weeks after injection clustering around the veins.[17, 27] After an injection of Thorotrast followed by an India ink injection, the more centrally located macrophages in the liver lobules segregate Thorotrast, while macrophages in the periphery of the lobules contain India ink.[27] This is interpreted as the migration of macrophages toward the central vein. However, Bernick, Hyman, and Paldino[12] after performing similar experiments, but using rats and Thorotrast and T-1824 dye, have concluded that the first phagocytosed colloids fill the macrophages and occlude sinusoidal lumens. These particles are then released and picked up by more centrally located phagocytes.

In young rats of the present experiments which retained injected Thorotrast from one to two weeks, macrophages showed no tendency to aggregate. Sinusoidal lumens are more tortuous and wider in young rats than in

those 30 days or older. Macrophages did not seem to occlude sinusoidal lumens as macrophages were evenly distributed in the liver lobules. This was not the case in rats older than 30 days, as phagocytes tended to be toward the periphery of the lobules. This corrobates the conclusions of Bernick, Hyman, and Paldino.[18]

(2) Spleen

Most of the phagocytic elements in the spleen are mobile cells, and the lining cells of splenic sinusoids seldom segregate this salt.[6, 8, 9] It is possible that, due to the complexity of the circulatory pattern of the spleen, the free histiocytes are able to engulf particulate matter before it reaches the fixed cells, thus limiting the functional potentialities of the latter. Since the splenic sinusoids are lined by fixed reticulo-endothelial cells, the organ is included in the section on fixed macrophages.

The spleen of the *17-day fetus* had a typical myeloid appearance, i.e., a predominance of hematopoietic red pulp. Normoblasts were distributed throughout the pulp, while lymphoblasts had not as yet appeared in the adventitia of arteries. No thorium-laden macrophages were seen.

At the *19th day* of gestation, the spleen was similar to the 17th day, except that lymphoid sheaths two to three cell layers thick appeared around the arteries. Phagocytosis occurred only in two out of 12 rats, and it was primarily limited to segregation of hemosiderin by free macrophages (Fig. 7). Stellate macrophages lining sinusoids were found occasionally.

Phagocytic activity is present in the spleen of chick embryos at 6 days incubation, and by the 9th to 13th day splenic macrophages ingest many more ink particles than do those of the liver.[11] The reticulo-endothelium of human fetuses begins its function as a macrophagic system at the end of three months, phagocytosis being more marked in the liver than in the spleen.[10, 20] The possible explanations of such contradictory findings, aside for species differences, are discussed in the section on the liver.

In the *21-day fetus* the white pulp was 3-4 cell layers thick in some areas, foreshadowing Malpighian bodies, while the red pulp contained many normoblasts and other hematopoietic elements. These blood cells made the sinusoidal linings difficult to observe. Of 7 fetuses, segregated thorium salt was found in two and hemosiderin in one; the other four did not contain either. Phagocytosis was restricted to the primitive red pulp and fixed macrophages were found only rarely.

Birth. Of 6 rats studied, Thorotrast was detected in only one animal in roentgenograms 3 hours after intracardiac injections. The particles of thorium salt in the one exceptional rat were flocculated primarily in the

tissue spaces and only a few globules had been segregated by free macrophages. The white pulp was now composed of 4-6 layers of lymphocytes.

Two days postpartum. At this time phagocytic activity was still not pronounced, even though the animals had been injected intracardially 24 hours before sacrifice.

Three days. The periarterial sheaths were no thicker than at birth. Rats given intraperitoneal injections showed few phagocytes in the red pulp; on the other hand, free macrophages were abundant in the red pulp of animals injected intracardially. Erythropoiesis was still active at this stage and many normoblasts were seen in the cords of Billroth and sinusoids.

At *5-7 days,* the periarterial sheaths were approximately 10 cell layers thick. Numerous free macrophages were in the cords of Billroth, many containing small amounts of round thorium aggregates. The sinusoidal walls were usually obscured but, when they could be observed, only a few littoral cells contained thorium particles. Intraperitoneally injected rats exhibited less phagocytosis than those injected intracardially. Normoblasts were still abundant.

Ten days. The periarterial sheaths were approximately 20 cell layers deep in some areas. Free macrophages with large vesicular nuclei were prevalent in the cords of Billroth and in the margins of the white pulp. This was interpreted as a migration of marginal lymphocytes into the red pulp and their transformation into macrophages.

15-20 days. Some of the arteries developed eccentric enlargements in their many-layered lymphocytic sheaths. The trabeculae and capsule became prominent. Although fixed macrophages were found, free macrophages were far more abundant. The sinusoidal walls were more distinct than in any of the earlier stages. Free macrophages were distributed primarily in the cords of Billroth (Figs. 8, 9), whereas the white pulp contained only a few. Nucleated red blood cells were still prevalent at the 20th postpartal day.

Rats injected either one or two weeks before sacrifice and those injected 8 or 24 hours before sacrifice showed no significant differences in amount and sites of the colloid segregated.

30 days. The number of normoblasts greatly decreased in this age group. Although sinusoidal lining cells were easily visible, most of the macrophagic activity was limited to free phagocytes in the cords of Billroth, as was the case in younger rats.

40-70 days. The spleen was histologically similar to the adult organ. Normoblasts have disappeared from the cords of Billroth by the 40th day. Connective tissue was well developed in the capsule and trabeculae, and the

central arteries of the Malphigian bodies were eccentrically placed. Macrophages were found primarily in the cords of Billroth, although some also appeared in the marginal portions of the white pulp (e.g. Fig. 8).

Comments. Phagocytic potentialities of the spleen were not expressed until late in the last third of gestation in the albino rat, and not until the 3d postpartal day did macrophages become abundant. Many filled macrophages were observed in the spleen by the 10th day and larger numbers by the 15th postpartal day. It is probable, therefore, that the reticulo-endothelium of the spleen reaches maturation at this period insofar as the phagocytic potentialities are concerned. Culbertson,[28] however, could not find differences in the phagocytic ability of spleen in rats ranging from 6 days postpartum to 60 days.

In general, the thorium salt is stored in free macrophages in the spleens of late prenatal, neonatal, juvenile, and young adult rats. Free macrophages continue to predominate in storing the colloid in adult rodents.[6, 9, 20]

(3) Lymph nodes

Unless otherwise noted, studies were made exclusively on nodes procured from animals sacrificed 24 or 48 hours after intraperitoneal injection. In general, many nodes in the rat show at least some thorium salt after intravenous injection; the collection in nodes increasing with time.[6] This is not true of mice as indicated by marked accumulation of the salt in widely distributed nodes. One explanation for this reaction is that the colloid is not permanently stored within the reticulo-endothelial cells but is in a state of continual redistribution.[9]

19-day fetus. The mediastinal and mesenteric nodes at this stage were incompletely formed. They were represented only by a fibrous capsule which enclosed a mesenchymal condensation within whose meshes free cells which were not phagocytic, occasionally appeared.

21-day fetus. The primordial mesenteric and mediastinal lymph nodes had differentiated into condensed masses of cells separated by sinusoids. The parenchymal cells still appeared mesenchymatous, but some contained thorium particles. Both types of nodes contained a few fixed and free macrophages (Fig. 10); however, more of the colloid was collected in the mesenteric than in the mediastinal nodes.

Birth-2 days postpartum. The mesenteric and mediastinal nodes differentiated into definite structural units with well-defined subcortical sinusoids. After intraperitoneal injection, Thorotrast was not detected in the diaphragmatic and substernal lymphatics by either histological or radiographic

methods (Figs. 1 and 14). Free and fixed macrophages were found in the subcapsular sinusoids of the nodes.

Third day. After intraperitoneal injection the substernal lymphatics and the lymphatics adjacent to the mediastinal nodes were filled with flocculated thorium salt (Figs. 2 and 15). Both free and fixed macrophages were more abundant in the periphery of the mediastinal nodes. In addition to mesenchymoid macrophages, a second type of phagocyte developed. This cell contained a large round, vesicular nucleus and abundant cytoplasm (Fig. 11) and was probably a lining cell which had freed itself from the reticulum.[4] The mesenteric lymphatics apparently became secondary in the rôle of absorption of material from the peritoneal cavity, since only a few free and fixed macrophages were recognized in the subcortical sinusoids of those nodes.

5-7 days. Cortex and medulla had not differentiated in the mediastinal nodes. Although thorium salt had flocculated in the subcapsular sinusoids, fixed and free macrophages were abundant. The mesenchymoid macrophages had disappeared and were replaced by another type of cell, possibly of lymphocytic origin, which had a small pycnotic nucleus and a small amount of cytoplasm. In addition, neutrophils were observed occasionally in the subcortical sinusoids, but these did not contain thorium-salt granules. Macrophages were still more concentrated in the peripheral portions of the node.

In contrast to the mediastinal nodes, primitive medulla and cortex were forming in the mesenteric nodes, which presented large sinusoids in the region adjacent to the hilus. Several concentrations of lymphocytogenic cells

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PLATE 3.

14. Peritoneal side of a diaphragm of a 2-day neonatal rat injected intraperitoneally with 0.06 cm^3 of Thorotrast. Note the lymphatic vessel is free of flocculated thorium and that the mesothelium appears flattened. x333

15. Peritoneal side of a diaphragm of a 3-day neonatal rat injected intraperitoneally with 0.035 cm^3 of Thorotrast. Note the submesothelial lymphatics are filled with flocculated Thorotrast. The nuclei of the mesothelium are rounded probably as a reaction to the colloidal injection. x333

16. Adrenal cortex of a 21-day fetus injected intra-embryonically with approximately 0.02 cm^3 of Thorotrast. This shows a fixed macrophage containing thorium globules. x575

17. Free macrophages in the subarachnoid space of a 15-day fetus injected intra-embryonically with approximately 0.01 cm^3 of Thorotrast. x333

18. Connective tissue macrophages in the mesenchyme of a 17-day fetus injected intra-embryonically with approximately 0.01 cm^3 of Thorotrast. x333

19. Kidney of a 20-day rat fetus injected intra-embryonically with approximately 0.02 cm^3 of Thorotrast showing flocculations of thorium salt globules in the glomerulus and an intertubular connective tissue macrophage containing thorium particles. x575

20. Kidney of a 20-day neonatal rat injected intravascularly with 0.15 cm^3 of Thorotrast showing tangential thorium salt casts in the thin loops of Henle. x333

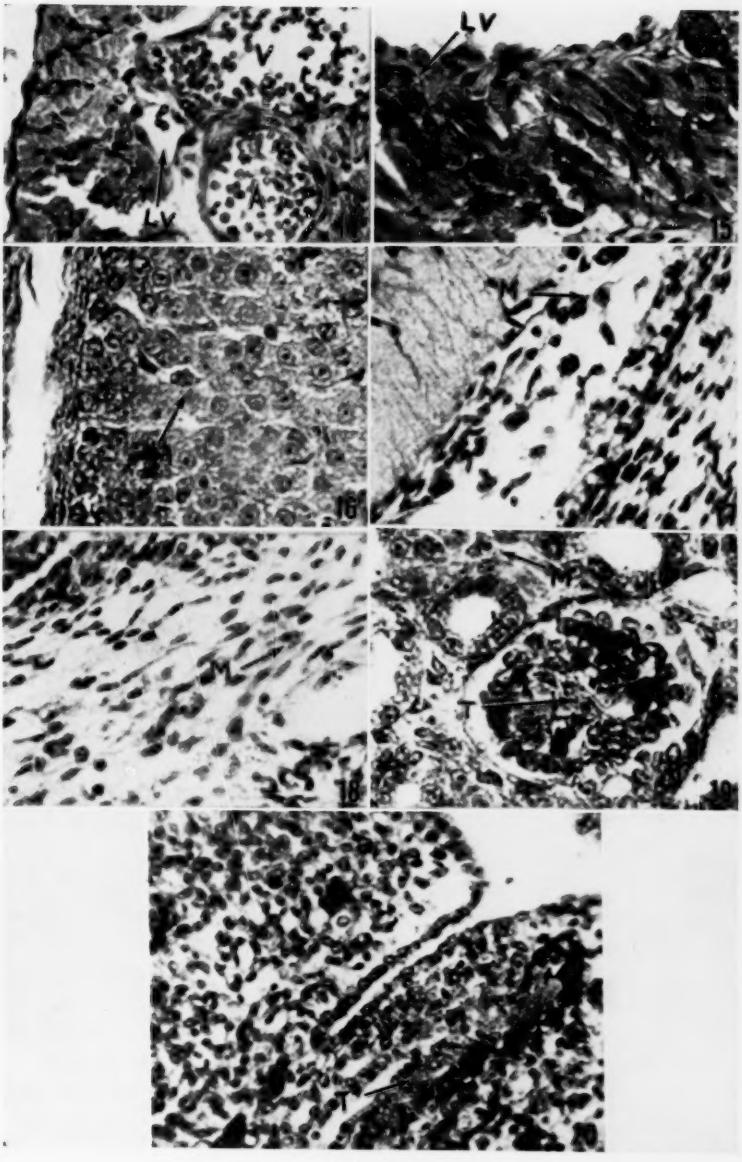


PLATE 3.

were evident in the cortex. This represented the first evidence of cortical lymph nodules. Large, mononucleated macrophages loaded with thorium salt were in the subcapsular and medullary sinusoids.

10-16 days. Phagocytic activity was similar to that of the 5-7-day rats. The nodes of the older group, however, were larger. In addition, mediastinal nodes were readily visualized by x-rays. Of rats killed one week after intraperitoneal injections totalling 0.50 cm^3 of Thorotrast per rat, marked histopathological changes appeared in the mediastinal lymph nodes. In one animal there was hyperplasia of the lymphatic nodules with complete disappearance of the reticulo-endothelium in some areas. Although macrophages laden with thorium salt were present, much of the colloid was in flocculated form. In the other two rats, fixed reticulo-endothelial elements were probably completely mobilized in both cortical and medullary sinusoids. Fibrogenesis occurred in those regions, as described by Harris[25]; the nodal parenchyma was replaced by connective tissue. He observed no marked hyperplasia of the lymphatic nodules and germinal centers.

A number of multinucleated giant cells appeared in the region of the sinusoids (Fig. 12; cf. Baillif,[4] Fig. 31). There were great variations in the size of these cells, amount of contained colloid, and number of nuclei. In general, the nuclei were round and vesicular, 2 to 25 per cell, and located peripherally. In most cells, plaques of thorium salt apparently had displaced the nuclei to one side, while in others small granules were present. The cytoplasm, wherever visible, was homogeneously eosinophilic. These cells appeared to be of the foreign body giant cell type similar to those described by Baillif,[3] and formed as early as one week after the first injection of Thorotrast. Giant cells, not of the foreign body type, formed in germinal centers of adult rats.[4]

The mesenteric nodes showed only a few macrophages. In animals injected with 0.1 cm^3 of Thorotrast, the nodes showed no histopathological changes one week after injection. The animals sacrificed 24-48 hours after injection had more macrophages mobilized than the former group.

By the *30th day* the mesenteric nodes were similar in structure to those of adult rats. The subcapsular sinusoids were much smaller, internodular sinusoids developed, lymphatic nodules formed, and medullary cords differentiated. The great majority of macrophages were large mononuclear fixed cells closely associated with the reticulum (Fig. 13).

The mediastinal nodes now showed the beginnings of differentiation into cortex and medulla, but lymph nodules were not yet developed. The subcapsular and medullar sinusoids were filled with flocculated thorium salt. A few free macrophages were present in those sinusoids, but greater phago-

cytic activity was evident in fixed reticulo-endothelial cells. The distribution of macrophages was, in general, similar to that of younger rat nodes. Some fixed macrophages were deep in the internodular sinusoids.

50-70 days. The mediastinal and mesenteric nodes were, at this age, identical with those of the adult. Macrophagic activity was similar to that of the 30-day animals.

(4) Adrenal

Phagocytosis was observed chiefly after intracardiac or intravenous injections, but of the animals injected intraperitoneally, only those given 10 cm^3/kg body weight showed evidences of phagocytosis. Therefore, observations were based primarily on animals given intravascular injections of Thorotrast.

21-day fetus. Only two out of 15 animals injected showed evidence of phagocytosis. The primitive cortical cells were separated by tortuous sinusoids extending from the periphery to the medulla. These sinusoids were lined with what morphologically appeared to be typical endothelial cells. Blood islands with occasional megakaryocytes were scattered throughout the gland. The few cortical macrophages present were located almost exclusively in the junction between the primordial zone fasciculata and zona glomerulosa (Fig. 16), and in the interlocking zone between the medulla and cortex. Some macrophages, however, occurred in the fascicular zone.

Birth-2 days postpartum. Radiations of the cortical sinusoids were becoming less tortuous. Blood islands were still present throughout the gland. Macrophages were more prevalent than in the fetuses, but their distributions were similar. Flocculated globules of the salt were occasionally present in the zona fasciculata.

5-7 days. Macrophages were mostly located in the medullo-reticular and glomerulo-fascicular junctions; distribution, therefore, was similar to that of the previous age group. Occasional blood islands and megakaryocytes were present until the 9th postpartal day.

15-40 days. In the cortex, the perimedullary sinusoids were wider than those in the previous age group. Cells lining the sinusoids had finely granular, eosinophilic cytoplasm with elongated, vesicular nuclei. Phagocytes containing some thorium salt were scattered through the medulla. These stromal macrophages were adjacent to the venous plexuses and in the adult rats were activated only after repeated injections.[6] In the present study most of the cortical macrophages were in the juvenile cortex, fewer in the fasciculo-glomerular junction and zona fasciculata in that order. The capsule contained an occasional macrophage.

50-70 days. With dosages of Thorotrast given (5 to 10 cm^3/kg body weight), macrophages were less numerous than in younger animals. In two rats the phagocytes were more numerous in the medulla and zona reticularis, while in two others they were more numerous in the zona fasciculata. Other rats in this age group showed little or no phagocytosis.

Comments. After repeated injections of Thorotrast into adult albino rats, thorium-laden macrophages appear in the sinusoidal walls of both the adrenal cortex and medulla, but stromal phagocytes occur principally in the medulla.[5] Sinusoidal macrophages were first activated in the cortico-medullary junction, then in the fasciculata, and later in the glomerulosa. Only after repeated injections were the stromal macrophages of the medulla activated. Baillif's[6] findings were in accordance with those of Wilson and Leduc.[27] Thorotrast injected into mice and followed by subsequent injection of India ink showed that ink particles were in the peripheral cortical cells, and thorium-laden cells in the deeper cortical regions. After a time, thorium-laden macrophages were no longer present in the cortex.[27]

B. Free Macrophages

The following is limited to a discussion of macrophages in the second subsystem of the reticulo-endothelium, i.e., the macrophages in the intercellular spaces of connective tissues. Macrophages freed from the sinusoidal walls have already been discussed with the fixed reticulo-endothelial elements.

(1) Free macrophages of fetuses

All fetuses between 11 and 14 days of age died when Thorotrast was injected. One 15-day-old fetus survived when injected with diluted Thorotrast and was sacrificed 6 hours later. In this animal, the colloid was accidently injected into the subarachnoid space. Free stellate-shaped macrophages were observed only in this space (Fig. 17). This is the earliest evidence of macrophagic activity in this study. By the last day of gestation free macrophages were present in various parts of the body (Fig. 18).

(2) Thymus

Free macrophages were in the vicinity of the gland from term fetuses to two-day rats, but since the capsule was not yet developed at this period, they were probably not associated with the gland per se. These mononuclear cells were round or oval in outline and possibly had arisen from lymphocytes which had migrated into the region.

By the 3d day postpartum, macrophages in the interlobular connective tissue of the gland were activated. They were spindle-shaped with elongated nuclei and resembled histiocytes of connective tissue. By the 7th day, typical connective tissue macrophages were present in the developing capsule.

Interlobular and capsular macrophages were found in older rats which had been injected intraperitoneally. These macrophages were either spindle-shaped or oval. In one 20-day animal, however, macrophages were seen in the lobules of the gland. These intralobular macrophages were located predominantly in the cortico-medullary junction, but a few appeared in the cortical and medullary substance as well. Their origin is unknown, but it is likely that these cells originated from the stroma of the gland. Baillif[1] reported that intralobular macrophages were present only after accidental involution following repeated intraperitoneal injection of colloids.

(3) Omentum

The greater omentum is of special interest since many macrophages can be mobilized within it from tâche laiteuses and histiocytes in the connective tissue. Intraperitoneally injected colloids enter the omentum and are picked up by the free macrophages.[1, 8]

Due to the relatively late development of the omentum, macrophages of this organ were not studied earlier than 8 days' postpartum. The omentum at that time contains little adipose tissue, its principal components being other connective tissue elements. After injections of Thorotrast, macrophages were abundant in its tissues. As the rats developed, more fat cells formed until they became the most conspicuous tissue element. Macrophages, probably of lymphocytic origin, were abundant in the outer zones of the tâche laiteuses. These observations in neonatal and young rats were in concordance with Baillif.[1]

(4) Testis

Free macrophages appeared in the connective tissue near the testis from the 6th postpartal day through the 70th day. Macrophages were located in the testicular capsule in only one animal which was 16 days old, and no macrophages were in the interstitial tissues. In intraperitoneally injected males, the continuity of the peritoneal cavity and the processes vaginalis was clearly visualized by x-ray.

After intratesticular injections of India ink into mice, Hooker and Pfeiffer[24] reported that "brown cells" in the intertubular spaces phagocytosed ink particles. These animals were pretreated with estrogens, and subsequently testicular tumors developed. Neither pretreatmnt with hormones and subsquent acute localized injections of colloids were given in the present

study; consequently, it was not possible to evaluate the findings with that of Hooker and Pfeiffer.

(5) Lung

Only occasional macrophages were in lung tissues. The first indication of the phagocytic potentiality of the septal cells was observed in several 20-day fetuses. In postpartal rats, macrophages were present only in those injected intravascularly. Most of the salt was in the form of small globules, but occasional plaques were observed. Macrophages were only rarely found lying free in the alveoli.

After Thorotrast injections, thoron X was reported in the feces and breath of rabbits and humans.[26] This indicates that at least isotopes of thorium were released. Furthermore, Easton[17] believes that one route of elimination of Thorotrast is the ascent of macrophages in the respiratory tract and passage in the feces or saliva.

(6) Kidney

The earliest evidence of thorium salt in the kidney was found in the 19-day fetus. There it appeared as small intraglomerular globules. Similar globules appeared in fetuses a day older, but in addition, free macrophages containing thorium salt were occasionally found in the perimalpighian connective tissue (Fig. 19).

Thorium plaques were primarily in the glomeruli and perimalpighian connective tissue macrophages in all stages of postpartal animals, these being most prominent in rats given intravascular injections. In one animal 20 days old, many thorium plaques collected in the thin limbs of Henle's loop as casts (Fig. 20). They appeared in such numbers as to occlude the lumen of the tubules. Such casts were reported by Baillif[1] in adult rats. Thorotrast is eliminated in great quantities through the kidneys beginning 34 days after the start of repeated Thorotrast injections and is associated with glomerular pathology, tubular casts, and decrease in urine volume.[21, 22]

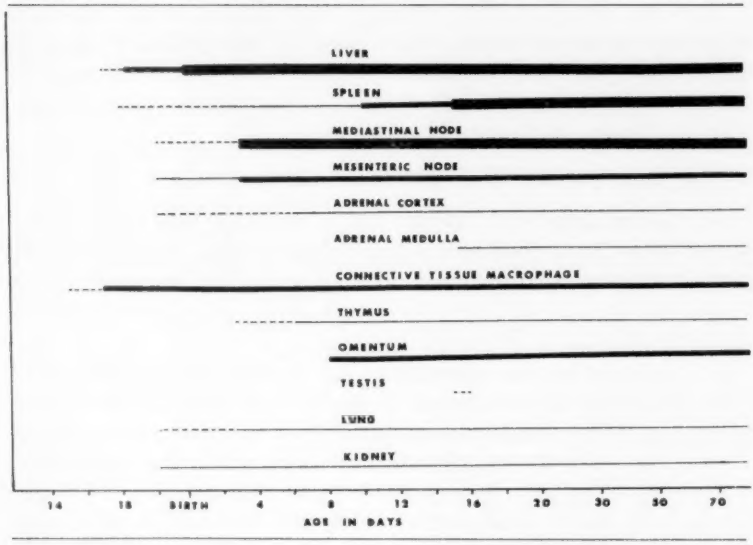
SUMMARY AND CONCLUSIONS

1. Six series of experiments *in vivo* were conducted for the study of phagocytic activity in albino rats of different ages. Of these, three were devoted to the study of fetuses, the remaining to postpartal rats. The animals were given a total of 5 to 10 cm^3/kg body weight of Thorotrast (colloidal thorium dioxide) and sacrificed at various intervals after injection. Tissues were removed and prepared for histological study; the smaller

fetuses were sectioned in their entirety. One hundred thirty-six fetuses and 138 postpartal rats were studied.

2. After intravenous injection of pregnant rats, Biebrich's scarlet, colloidal gold, or Thorotrast did not pass through the placentas into fetuses. X-ray placental visualizations were not obtained after Thorotrast injections.

TABLE 3. SEQUENTIAL MACROPHAGIC ACTIVITY IN THE ALBINO RAT. THE DOTTED LINES INDICATE OCCASIONAL PHAGOCYTIC ACTIVITY; THE SOLID LINES SOME ACTIVITY IN MOST ALL ANIMALS; THE HEAVY BARS GREATER ACTIVITY; THE HEAVIEST BARS, GREATEST ACTIVITY.



3. Thorotrast did not pass into fetuses through the skin, respiratory tract, or gastro-intestinal tract after intra-amniotic injection of Thorotrast.

4. Macrophagic activity in fetuses were demonstrated only after intra-embryonic injections of Thorotrast.

5. Table 3 summarized the time of onset of macrophagic activity and stages of sequential development of phagocytosis in various organs and tissues.

6. In general, by the 40th day the structural features and phagocytic function of the reticulo-endothelial system are similar to those of the adult rat.

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LESIONS IN "YELLOW" MICE FED STOCK, HIGH-FAT, OR HIGH-CARBOHYDRATE DIETS‡

"Yellow" mice such as those of strain YBR/Wi are represented by two genotypes which differ, as far as is known, in regard to one gene. Individuals possessing the Y′ gene are yellow coated and become obese when fed enriched diets; individuals not possessing the Y′ gene are grey coated and do not become obese when fed such diets.[5, 6, 25] In yellow-coated animals, food consumption and food utilization were found to be increased,[5, 15, 25] resistance to insulin to be increased,[25] but thyroid function to be unaltered.[20] Studies of endocrines failed to disclose differences in the structure of hypophysis, thyroids, adrenals, and testicles in yellows and non-yellows respectively,[11, 25] but yellows seemed to show decreased luteinization of the ovaries.[11]

In spite of this difference the grey- and yellow-coated animals appeared to be sufficiently similar from the genetic point of view for a study of the effects of obesity as such on such processes as skeletal growth and ageing and tumorigenesis, the nonobese littermates providing uniform control material for the obese animals. In the course of these investigations keratomalacia, panophthalmitis, osteoarthritis, hyperglycemia, and faulty skeletal development were observed,[10–22] changes that suggested the presence of severe metabolic disorders in both grey- and yellow-coated mice of this strain. Therefore, systematic studies of viscera were undertaken, the results of which are herewith presented.

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MATERIAL AND METHODS

Ninety-one grey male, 85 grey female, 79 yellow male, and 89 yellow female mice of strain YBR/Wi raised in our laboratory were from the time of weaning on put on (a) a stock diet of Purina laboratory chow, (b) a high-fat diet, and (c) a high-carbohydrate diet. These rations were fed *ad libitum* with water available at all times, and were well tolerated by the animals.

The main constituents of the diets, details of which were given previously,[19] were as follows:

Stock diet:		
Protein	26.2 gm	
Fat	5.4 gm	3.5 cal/gm
Carbohydrate	48.5 gm	
High fat diet:		
Base (Stock diet)	75.0 gm	
Lard (Swift's)	20.0 gm	4.6 cal/gm
Lactalbumen	5.0 gm	
High carbohydrate diet:		
Base (Stock diet)	60.0 gm	
Cornstarch	36.0 gm	3.7 cal/gm
Lactalbumen	4.0 gm	

The mice were weighed once a week and, if among the animals kept within a cage, individual weights varied more than 4 gm, individual weights were recorded. The animals were allowed to live to the end of their natural life, unless they had to be killed because of sickness. At necropsy, diseased tissues were saved for microscopic examination. In addition, sets of tissues were removed, irrespective of the presence or absence of gross findings. Details regarding the number of tissues examined are given in the tables summarizing the microscopic observations. The Chi-square test was applied to determine the statistical significance of some of the results obtained. The tissues were fixed in Bouin's solution and embedded in paraffin, sectioned and, as a rule, stained with hematoxylin and eosin. Methylviolet stains were made for the demonstration of amyloid; pituitaries were stained with Gomori trichrome, periodic acid Schiff, or Wolfe-Cleveland stain, and the pancreatic islets with Bensley's anilin acid fuchsin-methyl green, or Bell's chrome alum hematoxylin phloxine.

MICROSCOPIC FINDINGS

Cardiovascular system. There was aortitis distal to the valves, characterized by heavy infiltration with polymorphonuclear and mononuclear leucocytes (Fig. 1). In some instances, the process had spread to the myocardium (Fig. 2), the valves (Fig. 3), the mural endocardium and the pericardium, and fresh or organizing thrombi were found adherent to the aortic wall or the endocardium. Staphylococci and pneumococci were identified in spot checks of the exudate. Chronicity of the lesions was indicated by the

presence of fibroblasts and the development of a fibrillar connective tissue. The severity of the changes increased with advancing age, but the incidence was apparently not merely related to age. Grey-coated males—as old as yellow-coated females and older than grey-coated females—showed fewer lesions than either (Table 1).

Females of either coat color were more susceptible than the corresponding males. This difference was statistically significant, P being $< .02$ for the greys and P being $< .001$ for the yellows. Grey- and yellow-coated females were about equally affected.

TABLE 1. INCIDENCE OF AORTITIS AND MYOCARDITIS

Coat color	*Sex*	*No. of hearts examined*	*Mean age months*	*Aortitis and Myocarditis*	
				No.	*%*
grey	male	16	18.3	3	18.8
grey	female	43	17.5	25	58.1
yellow	male	37	16.5	5	13.5
yellow	female	44	18.3	24	54.5

Significance of sex differences in
grey males : grey females $P < .02$;
yellow males : yellow females $P < .001$.

In about 25 per cent of aged females and occasionally in an old male, the aortic wall had undergone calcification and cartilagination. Diet had no influence on the development or the course of the cardiac lesions. Deposits of varying amounts of amyloid were found in the hearts of animals of all groups (Table 7). Amyloidosis was not observed in mice younger than 12 months, and the incidence and severity increased with advancing age.

Respiratory system. Ageing mice showed bronchiectasis (Table 2) with foci of squamous metaplasia of the respiratory epithelium. Many bronchi were filled with homogeneous eosinophilic material or were lined by eosinophilic membranes, and varying amounts of lymphatic tissue were seen in the peribronchial layers. Bronchiectases were about twice as frequent in animals fed enriched diets as in stock-fed controls. This difference was, however, statistically not significant. Sex or coat color had no obvious influence on the development of these lesions.

Bronchitis and bronchopneumonia were rare in stock-fed mice but not infrequent in animals fed the enriched rations. In males of either coat color this difference was not statistically significant, but in females it was; in

yellow females it was highly significant P being < .001, and in grey females it was significant, P being < .02.

While in grey-coated mice no lung tumors were seen, adenomas were noted in 4 yellow-coated males and 4 yellow-coated females, 17 to 22 months of age (Table 8).

Spleen. Amyloidosis was common (Table 7). There was no case of systemic amyloidosis in which the spleen was not involved. Both nodular

TABLE 2. INCIDENCE OF PULMONARY LESIONS

						Pneumonia and bronchitis	
			Mean	*Bronchiectasis*		*Stock*	*Enriched*
Coat color	*Sex*	*No. of lungs examined*	*age months*	*No.*	*%*	*diet No.*	*diets No.*
grey	male	24	18.2	10	41.7	1	3
grey	female	57	19.1	21	36.8	2	9
yellow	male	37	18.6	10	27.0	1	3
yellow	female	60	17.3	28	46.7	7	19

Significance of dietary effects (stock and enriched diets) on bronchitis and pneumonia.
Stock : Enriched diet in grey females $P < .02$;
Stock : Enriched diet in yellow females $P < .001$.

and diffuse amyloid deposits were observed; frequently the infiltration was so severe as to obliterate completely the usual architecture (Fig. 6).

Liver. Occasionally fat vacuoles were noted in liver cells. In three mice, fresh infarcts and in two others abscesses were found. There was marked involvement of the liver in amyloidosis (Table 7; Fig. 8).

Altogether five hemangiomas and seven liver cell adenomas were found (Table 8). One of the latter was seen in a grey female 22 months old and kept on the stock diet; the remaining six occurred in yellow females. One of these 20 months old was fed the high-carbohydrate diet, and five had been kept on the high-fat ration. These mice were from 16 to 19 months old with a mean age of 17.2 months. Cirrhosis was not present in any of these animals.

Pancreas. The acini were unchanged. However, the epithelium lining small pancreatic ducts was flattened; many ducts were enlarged and filled with inspissated secretions (Fig. 10). In one grey male, fat necrosis, and in one grey female, acute pancreatitis were found. The most prominent

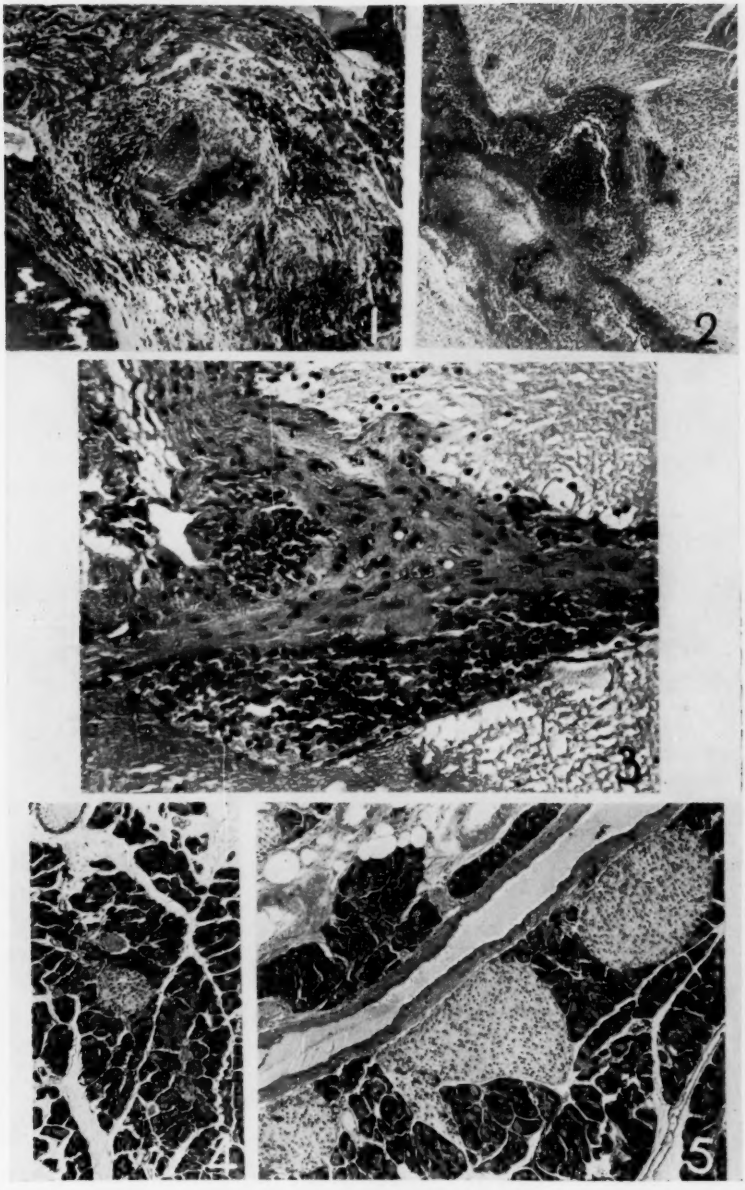

PLATE I

FIG. 1. Aortitis. Diffuse inflammation of aortic wall and narrowing of lumen. The dark irregular patch represents an area of cartilagination. x45.

FIG. 2. Myocardial abscess. x25.

FIG. 3. Aortic valve leaflet. Proliferation and leucocytic infiltration of endocardium. x240.

FIG. 4. Pancreas. Islet of regular size. x80.

FIG. 5. Pancreas. Hypertrophic and hyperplastic islets. x80.

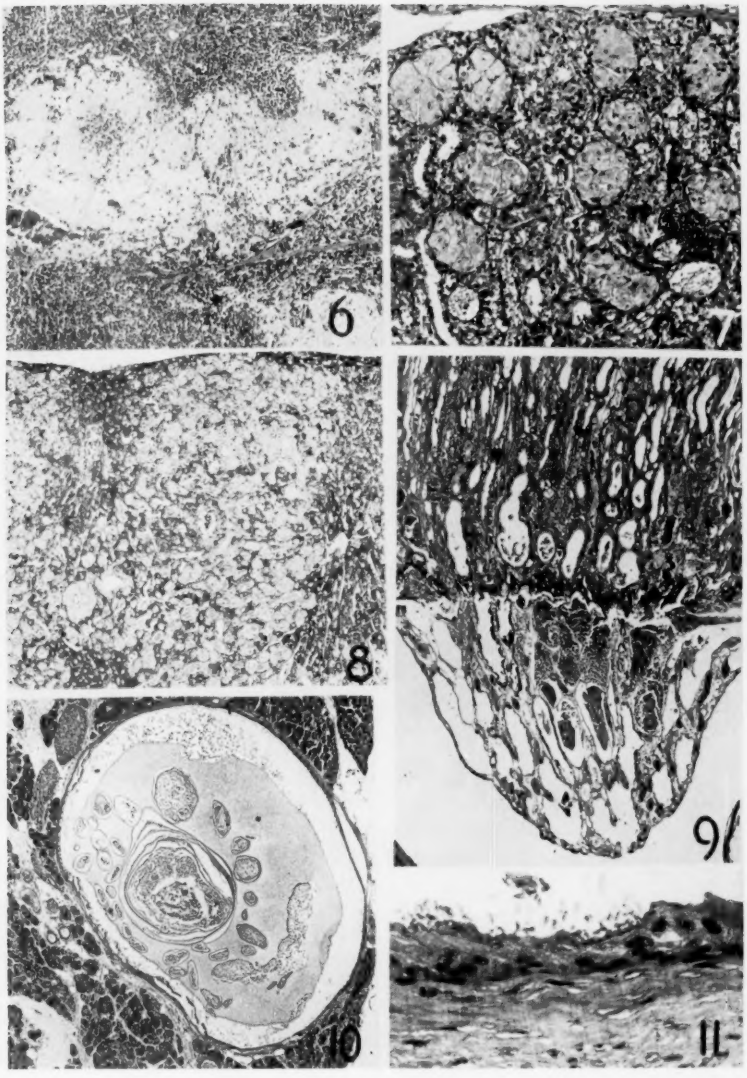

PLATE II

FIG. 6. Spleen. Amyloidosis. x80.
FIG. 7. Kidney. Amyloidosis of glomeruli. x100.
FIG. 8. Liver. Amyloidosis. x80.
FIG. 9. Kidney. Papillary necrosis; calcium deposits in zone of demarcation. x55.
FIG. 10. Cytic enlargement of pancreatic duct with concretions. x33.
FIG. 11. Squamous metaplasia of urethral epithelium. x490.

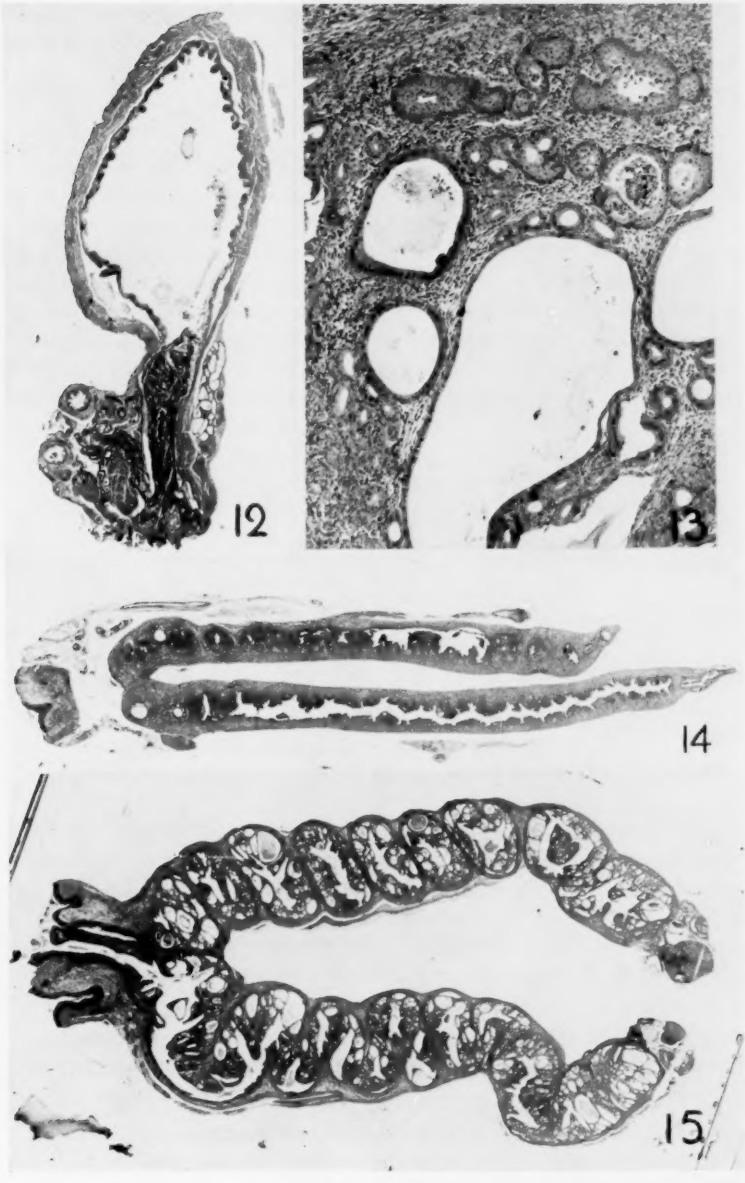

PLATE III

FIG. 12. Urinary bladder and urethra. The latter is distended by plug of debris. x8.

FIG. 13. Focus of squamous metaplasia in hyperplastic endometrium. This area was located at a considerable distance from the cervix. x60.

FIG. 14. Uterus of regular size and configuration. x6.

FIG. 15. Uterus showing marked cystic hyperplasia of endometrium. x6.

findings were hyperplasia and hypertrophy of the islets of Langerhans (Figs. 4, 5). Differential stains for cytoplasmic granules failed to show consistent deviations from the usual pattern. Ordinarily the diameter of the pancreatic islets of mice of various strains is about 75 to 100 μ. Islets measuring 250 to 300 μ were not infrequent, and coalesced islets had an aggregate diameter of as much as 600 μ. Somewhat arbitrarily a diameter of 175 μ was chosen as indicating conspicuous hypertrophy. Enlarged islets were seen in animals 9 months of age and older. Irrespective of the diet consumed yellow-coated males had the highest incidence of enlarged islets

TABLE 3. INCIDENCE OF "GIANT" PANCREATIC ISLETS

			Giant pancreatic islets		
Coat color	*Sex*	*No. of pancreas examined*	*No.*	*% of total*	*% in mice on high-carbohydrate diet*
grey	male	23	8	34.8	70.0
grey	female	52	25	48.1	61.5
yellow	male	33	22	66.7	93.8
yellow	female	50	24	48.0	56.3

Significance of differences:
Irrespective of diet—Grey males : yellow males $P < .05$;
High-carbohydrate diet—Yellow males : yellow females $P < .05$.

(66.7 per cent). The difference between grey-coated and yellow-coated males was statistically significant, P being $< .05$. Feeding of the high-carbohydrate diet increased the incidence of "giant" pancreatic islets in animals of all groups but particularly in males. This difference in the response to the high-carbohydrate diet was statistically significant if yellow-coated males and yellow-coated females were compared, P being $< .05$. (Table 3).

Adrenals. Three mice showed cortical hyperplasia. These animals were scattered over various groups. Amyloid involvement was common and reached considerable proportions (Table 7).

Kidneys. Pyelonephritis was frequent (Table 4). In acute stages, the tubules were filled with polymorphonuclear leucocytes, and microabscesses were found in the cortex and throughout the medulla. In chronic stages, there was irregular scarring of the surface with partial or complete hyalinization of glomeruli, dilatation of tubules, and fibrosis of the medulla. There was focal or diffuse infiltration by mononuclear leucocytes. As a rule, the medullary portion was more severely involved than other segments, and

hyaline casts in enlarged tubules gave rise to a thyroid-like appearance of the organ. Papillary necrosis with deposits of calcium in necrotic areas was not uncommon (Fig. 9) and associated with a more or less advanced degree of hydronephrosis. Under these conditions the pelvic epithelium was flattened or had undergone squamous metaplasia. The incidence of papillary necrosis was somewhat higher in grey-coated than in yellow-coated animals (Table 4). However, these differences were not statistically significant. Diet did not exert any influence on the development of these lesions. Besides these inflammatory and regressive changes, there was thickening of the

TABLE 4. INCIDENCE OF KIDNEY LESIONS

				Pyelonephritis				
						associated with		
Coat color	*Sex*	*No. of kidneys examined*	*Mean age months*	*No.*	*%*	*papillary necrosis No.*	*amyloid in kidney No.*	*amyloidosis generalized No.*
grey	male	27	18.6	26	96.3	6	10	21
grey	female	48	19.6	46	95.9	16	15	36
yellow	male	39	16.5	36	92.3	6	12	25
yellow	female	63	18.9	59	93.7	7	13	41

basement membranes of the glomerular tufts and deposition of hyaline material simulating intercapillary glomerulosclerosis. Special stains, however, disclosed the amyloid nature of these changes. Small amyloid deposits were also found in the interstitial tissue between the tubules. While all mice affected with amyloidosis had pyelonephritis, the opposite did not hold good: many mice showing pyelonephritis failed to show amyloidosis in the kidney (Table 7). Papillary necrosis on the other hand was in all but one case associated with renal amyloidosis (Table 4).

Urinary bladder and urethra. In about 10 per cent of all mice the mucosa was infiltrated by polymorphonuclear and mononuclear leucocytes with focal loss of the epithelium, squamous metaplasia (Fig. 11), and the formation of homogeneous or calcified debris (Fig 12). In males, the urinary bladder was often considerably distended with urine and reached above the umbilicus. The urethra of these mice as well as of those showing less marked distention frequently was dilated and the lumen clogged with debris (Fig. 12). The transitional epithelium had undergone squamous metaplasia of varying degree (Fig. 11).

Prostate. In old mice the dorsal as well as the ventral prostatic acini were increased in number and size, and small nodules were found. Littre's glands showed marked hyperplasia and extended far into the surrounding muscle tissue; slight inflammatory reaction was not uncommon in the area. The enriched diets had no appreciable effect on the development of the prostatic changes (Table 5).

Testicles. No noteworthy changes were encountered.

TABLE 5. INCIDENCE OF HYPERPLASIA AND HYPERTROPHY OF THE PROSTATE

Coat color	*No. of prostates examined*	*Mean age months*	*Prostatic hyperplasia and hypertrophy*	
			No.	*%*
grey	25	17.7	10	40.0
yellow	39	16.3	13	33.3

TABLE 6. INCIDENCE OF ENDOMETRIAL HYPERPLASIA

Coat color	*No. of uteri examined*	*Mean age months*	*Hyperplasia of endometrium*	
			No.	*%*
grey	46	19.7	36	78.3
yellow	55	18.7	39	70.9

Uterus. The uteri of many females of both coat colors were enlarged, thickened, and reddened. The endometrium was hyperplastic and hypertrophic. The glands were closely packed, often cystically dilated, and lined by tall secreting columnar epithelium (Figs. 13, 14). The latter showed foci of squamous metaplasia (Fig. 12). Occasionally there was slight mononuclear cell infiltration. The incidence of these hyperplastic and hypertrophic changes increased with advancing age (Table 6), and rose to 100 per cent in mice older than 16 months.

Adenocarcinoma was seen in one grey mouse, 18 months old. Here and there small myomatous seedlings were present in association with endometrial polyps. Myosarcomas were found in two yellow-coated females, 18 and 20 months old respectively. Almost all uteri showed epidermization of cervical glands.

Ovaries. In both grey-coated and yellow-coated animals, considerable amounts of lutein tissue were seen, while follicles were small and limited in

number. In old mice telangiectases were found in the stroma, and small cysts lined by cuboidal epithelium.

Mammary glands. In aged females the ducts were dilated and filled with homogeneous material. The lining epithelium was flattened, and there was no evidence of growth activity.

Thyroids. The structure of the glands varied. Even in young mice clusters or nodules or dilated acini lined by flattened epithelium alternated

TABLE 7. INCIDENCE AND DISTRIBUTION OF AMYLOID

			Amyloidosis : % of tissues examined				
Coat color	*Sex*	*Diet*	*Spleen*	*Liver*	*Heart*	*Adrenal*	*Kidneys*
grey	male	Stock	90	88	60	56	56
		Fat	0	0	0	0	0
		Carbo.	90	62	60	75	38
grey	female	Stock	88	13	18	20	0
		Fat	58	38	53	0	27
		Carbo.	88	79	82	40	58
yellow	male	Stock	88	22	16	9	29
		Fat	17	0	0	0	0
		Carbo.	88	72	50	67	41
yellow	female	Stock	89	10	20	0	14
		Fat	75	14	27	0	6
		Carbo.	90	70	56	30	39

Significance of the dietary effects on amyloidosis:
Grey males Stock diet : High-fat diet $P < .001$;
Yellow males Stock diet : High-fat diet $P < .05$.

with normal-looking ones. Inactive follicles filled with firm colloid increased with advancing age. There was no evidence of stimulated activity.

Hypophyses. There was no consistent change in the distribution of the various cell types. In old animals, the number of chromophobes was increased; eosinophiles were present in usual numbers; basophiles were conspicuous, and in some instances they were degranulated.

Amyloidosis. The distribution of the lesions is shown in Table 7. The deposits varied from scattered foci to diffuse infiltration with obliteration of the architecture of the tissue affected (Figs. 6-8). The spleen was most frequently involved, with the liver following in frequency; myocardium,

adrenals, and kidneys were about equally affected and third in line. In addition lymph nodes, stomach, uterus, and other organs occasionally showed amyloid infiltration. Amyloidosis was not seen before the end of the first year of life, and it increased in incidence and severity with advanc-

TABLE 8. INCIDENCE OF TUMORS AND CYSTS

Type of lesion	*Grey male*	*Grey female*	*Yellow male*	*Yellow female*
Lipoma in groin	1			
Adenoma of lung			4	4
Mastocytoma of stomach and pancreas		2	4	
Adenoma of liver		1		6
Angioma of liver		2	1	2
Myoma of uterus		1		
Mesenteric cysts		3		11
Papillary carcinoma of lung		1		
Adenocarcinoma of uterus		1		
Papillary carcinoma of urinary bladder		1		
Squamous cell carcinoma of parotid				1
Myosarcoma of uterus				2
Sarcoma of bone*				3

*1 periosteal fibrosarcoma of clavicle
1 osteogenic sarcoma of clavicle
1 osteogenic sarcoma of rib

ing age. There was a strong positive correlation (95 to 100 per cent) between amyloidosis and pyelonephritis (Table 4); however, the severity of the kidney disease did not correlate with that of amyloidosis: advanced pyelonephritis as well as papillary necrosis was sometimes associated with only slight or minimal amyloid deposition.

In males fed the high-fat diet the incidence of amyloidosis was decreased, this difference being highly significant ($P < .001$) in grey-coated males and significant in yellow-coated males ($P < .05$). In addition, the lesions were

less severe in the females after feeding the high-fat diet. This effect was, however, not consistent and statistically not significant. The high-carbohydrate diet on the other hand tended to increase generalized involvement and the severity of amyloidosis in animals of all groups, although the statistical significance varied from 0 to $P < .01$ (Table 7).

Tumors and cysts. The neoplasms and the cystic lesions are tabulated in Table 8.

DISCUSSION

In mice of strain YBR/Wi, aortitis, bronchiectasis, pyelonephritis, hypertrophy of the pancreatic islets, endometrial and prostatic hyperplasia, and generalized amyloidosis were observed. Squamous metaplasia with inspissated secretions was seen in the pancreatic ducts, respiratory epithelium, urinary tract, and uterus.

Pulmonary, vascular, renal, pancreatic lesions, and generalized amyloidosis occurred in males and females, in animals of either coat color, and irrespective of the diet consumed. However, the distribution of the lesions was unequal in the various groups, and there were some variations in the incidence of certain lesions within certain groups: Animals of both sexes were equally prone to acquire kidney disease, bronchiectasis and, if fed the stock diet, amyloidosis. Females were significantly more susceptible to cardiovascular disease than males. Sex differences were also noted in the response to different dietary regimens; under the influence of a high-fat diet females (grey-coated as well as yellow-coated) developed more lung disease than males, while males showed a more distinct attenuation of amyloidosis than females. A high-carbohydrate diet on the other hand was more effective in males than in females in accentuating the hypertrophy of the pancreatic islets.

Differences associated with coat color were not striking. Yellow-coated males tended to develop giant pancreatic islets more readily than grey-coated controls. However, this difference was less conspicuous after consumption of a high-carbohydrate diet which affected grey-coated males more than yellow-coated controls. Yellow-coated animals developed a larger crop of tumors, although the total number of neoplasms was not large in either group.

Many of the findings, such as squamous metaplasia of columnar epithelium in various locations, support the suggestion based on previously reported observations in the eyes[19] and on faulty skeletal molding[21] that mice of this strain may suffer from a relative vitamin A deficiency.

Although mice of different strains are known to vary in their nutritional requirements,[15, 16] it is not clear why mice of strain YBR/Wi should develop vitamin A deficiency on rations that are adequate for mice of other strains. Since the present investigations are of morphological nature and since only a few physiological studies have been carried out in mice possessing the Y′ gene, any attempted correlation of morphological changes and pathophysiological conditions will have to be tentative and will have to await confirmation. Still, the diverse morphological changes observed point to the presence of an integrated pattern, missing links of which will have to be furnished by further morphological and by biochemical and biophysical studies.

In the search for possible causes of a relative vitamin A deficiency it should be noted that there is in mice of strain YBR/Wi a tendency to hyperglycemia as evidenced by spot checks of blood sugar levels which we found to be as high as 300 mg%. This is in agreement with the increased insulin resistance noted in these mice by other observers.[25] Disturbances in carbohydrate metabolism on the other hand may be associated with disturbances in the conversion of carotene to vitamin A.[2, 17] Such conditions might account for a relative vitamin A deficiency in mice of strain YBR/Wi. Carotene conversion is furthermore decreased in hypothyroidism.[2, 17] The thyroids of "yellow" mice present a microscopic structure that is consistent with a low level of activity. This in turn may add to a defect in carotene conversion. Another contributory factor may be operating in YBR/Wi females which, irrespective of coat color, developed considerable amounts of lutein tissue in the ovaries. The latter presumably produce large quantities of lutein which in turn decreases the storage of vitamin A by the liver.[9]

Increased susceptibility to infection might be expected in vitamin A deficiency; one might therefore expect a certain parallelism between infection and changes directly attributable to this deficiency. Such a combination was noted in females of both coat colors which showed a more marked retardation of skeletal molding[21] and a higher incidence of inflammatory cardiac lesions than males. Cardiovascular disease is uncommon in mice, and special conditions may be necessary for the development of such changes.

No sex differences were present in regard to the kidney lesions. Chronic pyelonephritis is common in old mice and it is obviously not related to any vitamin deficiency. Pyelonephritis was often associated with papillary necrosis. Lesions of the latter type have been observed in mice of strain A, where they were thought to be secondary to primary renal amyloidosis.[9] In mice of strain YBR/Wi, the relationship between pyelonephritis and

amyloidosis is apparently not the same as in mice of strain A, since in the former pyelonephritis was twice to four times more frequent than amyloidosis. Furthermore, there was no correlation between the amount of amyloid deposited and the severity of pyelonephritis. While thus in especially severe renal amyloidosis amyloid may have contributed to the development of pyelonephritis, the latter must have additional causes such as urinary obstruction by the masses of debris found in the urethra.

The numerous deposits of amyloid in the cardiovascular system might suggest that the amyloidosis is primary in character. However, the general distribution of the infiltration does not exclude the possibility of secondary amyloidosis, especially since the spleen and liver were even more prominently involved than the heart; this holds good not only as far as the number of mice is concerned, but also regarding the amount of amyloid deposited. The possibility that one is dealing with secondary amyloidosis is supported by the beneficial effect of the high-fat diet which was conspicuous in all groups except grey females and present to some extent also in the latter. The fat used in the enriched diet was lard which contains a factor acting like vitamin A.[12] In the presence of this vitamin-A-like substance the course of infections would be attenuated and beneficial effects on secondary amyloidosis might be expected. However, the nature of amyloidosis is not adequately understood, and eventually the distinction between primary and secondary amyloidosis may turn out to be untenable.

The question arises whether or not the pronounced amyloidosis occurring in this strain of mice is related in some way to the peculiar endocrine make-up of these animals. Spontaneous amyloidosis occurs in strains of mice in which there is no apparent evidence of endocrine imbalances. On the other hand, experimental amyloidosis produced by injections of sodium caseinate was influenced by sex,[14] and markedly increased under the influence of cortisone or ACTH, and to some extent also by castration or thyroidectomy.[13, 24] As indicated by the insulin resistance of yellow mice and as will be discussed subsequently, there are indications of hypophyseal dysfunction in these animals. Whether or not ACTH is involved in this imbalance is a moot question, but there is at least no evidence to the contrary. Conceivably therefore in mice of strain YBR/Wi, an increased tendency to infections conditioned by disorders in the metabolism of vitamin A is associated with a hormonal pattern favoring the deposition of amyloid. Thus may be brought about the high incidence of amyloidosis in these mice.

The endocrine make-up of strain YBR/Wi is unusual, although individual features of this pattern have been noted in mice of other strains. The

structure of the thyroid suggests fluctuation in activity, and systemic evidence of hypothyroidism was indicated by faulty skeletal development[21] and low energy requirements in these mice.[5, 15, 25] The marked luteinization observed in the ovaries may account for the endometrial hyperplasia. Increased luteinization of ovaries was also seen in mice made thyroid deficient with I^{131}; moreover, cystic uterine dilatation or hyperplasia was seen in radiothyroidectomized mice carrying transplanted hypophyseal tumors.[7] Therefore, the changes occurring spontaneously in thyroid, ovaries, and endometrium of mice of strain YBR/Wi may not be a mere coincidence but may be interrelated and parts of a definite endocrine pattern.

The conspicuous hypertrophy of the pancreatic islets, present in mice of strain YBR/Wi has also been observed in hyperglycemic obese mice (Obob) in which the basic metabolic disturbance was found in a block of CO_2 fragment oxidation and of the hexokinase reaction.[1] It would appear justified to consider the hypertrophy of the pancreatic islets as secondary to some other functional or morphological disturbance present in mice of strain YBR/Wi, such as hypophyseal or hypothalamic dysfunction resulting in hyperglycemia.

Tumors were rare. Of those occurring with increased frequency the lung tumors and mastocytomas are probably carry-overs from the strain A ancestry of this strain.[8, 14, 19] Osteogenic sarcomas are known to occur in "yellow" mice.[9, 25] The fact that five of seven hepatomas were found in yellow-coated females fed the high-fat diet would appear noteworthy, although the findings are statistically not significant. Moreover, no evidence of other dietary deficiencies besides that of vitamin A was obtained—there were no fatty livers in particular—and such neoplasms are seen also in "yellow" mice fed regular diets.[28] The occurrence of a single adenocarcinoma of the endometrium indicates that the endometrial hyperplasia which was so frequent in these mice did not play a major rôle as a precancerous condition.

SUMMARY

In obese and nonobese mice of strain YBR/Wi, squamous metaplasia of the respiratory and urinary epithelium, of the epithelium of the pancreatic ducts, and the endometrial glands was recorded. These changes in association with keratomalacia and retarded skeletal molding indicate a disturbance in the metabolism of vitamin A, probably with involvement of the thyroid. The frequent occurrence of aortitis and myocarditis is interpreted as signs of lowered resistance to infection. A high incidence of systemic

amyloidosis, attenuated by a high-fat diet, was observed. Hyperplasia and hypertrophy of the islets of Langerhans was seen and was particularly frequent after consumption of a high-carbohydrate diet. Endometrial hyperplasia associated with marked luteinization of the ovaries was a common finding in aged females. The relationship of the visceral lesions to the endocrine pattern and the metabolic disturbances observed in mice of strain YBR/Wi is discussed.

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EVENTS

YALE MEDICAL SOCIETY

January 14, 1957

ON THE INCORPORATION OF NUCLEOTIDES INTO RIBONUCLEIC ACID BY SOLUBLE MAMMALIAN ENZYMES. By E. S. Canellakis, Department of Pharmacology.

The isolation of a soluble rat liver enzyme preparation which incorporates C^{14}-labeled uridine monophosphate and adenosine diphosphate into a large molecular weight polymer was described. Evidence was given that this polymer has the properties of RNA. The microsomes of a rat liver homogenate contain a factor which inhibits this incorporation. This enzymatic system affords a means for the study of the mechanism of nucleic acid synthesis by mammalian tissue.

E. S. CANELLAKIS

COARCTATION OF THE AORTA IN INFANCY. By Ruth Whittemore and Marjorie Fouts, Department of Pediatrics.

Twenty-two of forty-six consecutive cases of coarctation of the aorta in infants, seen over a ten-year period, have survived to date. Early onset of symptoms proved a bad prognostic sign; all twenty-four who died had shown severe difficulty prior to six weeks of age and expired before six months of age. Congestive heart failure was by far the leading cause of death, and, especially in patients with accompanying endocardial fibroelastosis, bouts of paroxysmal auricular tachycardia often signaled the terminal event. At post-mortem a ductus arteriosus was patent in all but two children. The ductus appeared distal to the coarctation in eighteen, near or entering it in six, but never proximal to the stenotic segment. Interestingly, three out of five with a fulminating course, two others and a sixth suspected but not examined at post-mortem, showed an associated subendocardial fibroelastosis.

While cardiac failure and cyanosis were the most common presenting complaints in those who did not survive, poor weight gain was the most common initial cause for investigation in those still living. In general, the surviving patients, all but one of whom have been followed for over three years since diagnosis, showed a clinical picture of slow initial weight gain and development followed by some improvement as well as other classical signs of coarctation. In contrast to one previous report by others no one electrocardiographic pattern of ventricular hypertrophy correlates well with either clinical course or prognosis.

Successful medical management of twenty-two of forty-six cases suggests that surgery should be reserved for specific indications. These include inability to maintain compensation, lack of growth, electrocardiographic evidence of left ventricular hypertrophy, increasing blood pressure, increasing heart size out of proportion to general growth, aortic insufficiency or other

associated difficulties. Only two of the present series went to surgery; one died during anesthetic induction and the other, forty-eight hours post-operatively.

R. I. B.

February 4, 1957

CARDIORESPIRATORY CHANGES AT BIRTH. By Virginia Apgar, Department of Anesthesiology, Columbia University College of Physicians and Surgeons, New York, New York.

The period of greatest infant mortality is from the onset of labor to 48 hours after birth. The key to reduction of fatalities during this period is an expanded knowledge of cardiorespiratory changes at birth. A certain amount of work has been done in animals, but little is known at the present of changes in human infants, and exploration in this field is just beginning. For example, one recently discovered fact is that the systolic pressure in the pulmonary artery of newborn infants remains between 50-60 mm. of Hg for the first two weeks after birth before dropping to the normal adult pressure of 12-30 mm. of Hg. Clinical information on pathological states in this period is similarly growing. As an example, it is found that the optimal time to expand the lungs of all premature infants without causing damage is at birth, using a pressure of 40-50 cm. of water. Gastric intubation for oxygen therapy is illogical. New techniques include such items as umbilical vein catheterization.

N. R. M.

ZOOLOGICAL JOURNAL CLUB

February 6, 1957

ECOLOGY OF RANA PIPIENS. By Jack B. Bresler, Chairman, Department of Biology, Bard College, Annandale-on-Hudson, New York.

Twenty-nine measurements were made on 813 specimens of *Rana pipiens* from different geographic regions of Canada, the United States, and Mexico. These findings were compared with 14 environmental factors found in the areas to see if there was any correlation. The majority of specimens was from areas with an annual temperature of 60° F. and 20-60 inches of precipitation yearly.

The highest correlations are found between size and temperature and size and precipitation; the lowest between size and geographic origin. Body size increases with temperature, but reaches an optimum, falling off at temperatures higher than 75°-79° F. Most of the other linear measurements vary directly with over-all body length. Amount of spotting on the back and on the tympanum is related to temperature and altitude, but spotting on the eyelids and on the head is unrelated to any environmental factor. Phenotypically very similar frogs are found above 6,000 feet in Mexico (active 12

months of the year) and below 1,000 feet in Illinois (active only 3 months). Therefore, further taxonomic separation of geographic species or subspecies is impossible.

G. E. W. 3RD

NEUROLOGICAL STUDY UNIT

February 12, 1957

MYASTHENIA GRAVIS. By Paul F. A. Hoefer, Department of Neurology, Columbia University College of Physicians and Surgeons, New York, New York.

Myasthenia gravis is a disease characterized by muscular weakness and abnormal fatigability without any related muscular pathology. Bulbar symptoms such as diplopia, inability to move the eyes, weakness in swallowing, dysarthria, and others are especially noticeable. It is believed that this condition is due to some sort of interference with transmission across the myoneural junction since it mimics curare poisoning and is aided somewhat by anti-cholinesterase therapy; however, the nature of this block is not understood. The disease predominates in women, and it can occur at any age, although it is milder in children.

Various correlations have been made between myasthenia gravis and epilepsy, the collagen diseases, hyperthyroidism, and others, but perhaps the most notable one is thymoma. Fifteen to twenty per cent of myasthenia gravis patients have an associated thymoma, and they also have a more severe form of the illness. Remissions following thymectomy have been reported in 55 per cent of young women by one group of investigators. This, however, has not been confirmed by others. The prognosis of patients with thymoma is grave. Most investigators have not seen remission following thymomectomy. It should be noted, however, that only patients with a good chance of survival are operated upon, and also that figures state that 35 per cent of all myasthenics will show spontaneous remission with only drug therapy.

Attempts to reverse weakness with anti-cholinesterases have been made with some success. Agents in common use are Prostigmine, Pyridostigmine, Mestinon, Tensilon, TEPP, and DFP. A newer drug, octamethylpyrophosphoramide (OMPP), is longer acting, but it is also extremely toxic. Atropine is used to overcome various toxic effects and Tensilon to reverse the components of the so-called "cholinergic crisis." Various hormones have been tried but with inconclusive results.

The main problem with this disease is that about 30 per cent of myasthenics die even when they are receiving "rational therapy." The first two years are the most dangerous, and the mean life span for those with continuous illness is eight years. Often a minor respiratory infection initiates a myasthenic crisis during which time the patient remains apneic and is refractive to all medication. Patients have even died in respirators for no apparent reason. Further research should clear up the latter and many other problems of myasthenia gravis.

J. A. O'N.

SIGMA XI LECTURE

February 13, 1957

RADIOACTIVE FALLOUT. By W. F. Libby, Fermi Institute of Nuclear Studies and Atomic Energy Commission.

There are different types of fallout, containing different mixtures of radiations, but only those posing ingestive hazards are here considered. Of these, strontium 90, because of its high yield and its chemical similarity to calcium, is the most important.

Local fallout settles rapidly, while the tropospheric debris may require a month to settle and gains a world-wide distribution along broad latitudinal bands. The stratospheric fallout, produced only by weapons in the megaton range, may not settle for up to ten years and achieves a global pattern of distribution. The fact that fallout of the latter two types is minimal in desert regions and virtually undetectable in regions of no rainfall suggests that atmospheric water is the primary precipitating agent.

Once in the topsoil, Sr is absorbed by plants, though it is discriminated against in favor of Ca by a factor of about 1.4. In very acid soils, as those of Wales, this factor may be reduced to one. Milk producers eating this grass discriminate further by a factor of about seven, while the milk consumers do so by one of from two to eight. In the topsoil of the United States today there are, on the average, 50 micromicrocuries of Sr 90 per gram of available Ca, which represents one-half the maximum permissible level in individuals as determined by the Atomic Energy Commission. The amount present within the bodies of the inhabitants of this region is only one-hundredth of this limit. Even in the absence of further tests, however, it may rise to two to three times the present level since there is sufficient Sr 90 in the stratosphere to maintain current topsoil levels for 30 years.

Of all the radiations to which the body is subjected, cosmic radiations seem physically most similar to those produced by Sr 90. Presumably, this indicates their effects are the same. Thus, it can be shown that the present Sr 90 level in children is equivalent to the extra cosmic radiation received from an altitudinal increase of 300 feet.

While a sense of false security is to be avoided, it does appear that Sr 90 poses no immediate hazard. Fallout must be studied, however, for an understanding of its effects are essential for the peacetime use of atomic energy.

P. K.

SIGMA XI DISTINGUISHED VISITOR LECTURE

February 14, 1957

RADIOCARBON DATING. By W. F. Libby, Fermi Institute of Nuclear Studies and Atomic Energy Commission.

Collision of cosmic rays with the atmosphere produces neutrons whose density reaches a maximum just below the top of the atmosphere. Most of these neutrons eventually form carbon-14 out of nitrogen-14. This rate of

C^{14} production is apparently constant in time, but varies with latitude. Oceanic and atmospheric mixing and frequent recycling of the atoms through the biosphere result in an empirically uniform distribution over the earth's surface. Since the C^{14} is transformed into CO_2, it is assimilated by living organisms and should reach an equilibrium density, which is known and is constant. Beginning at the time of death of the organism, the C^{14} content will decrease because no new C^{14} is being added and half the amount present is lost every 5600 years. Thus, a method for dating time when carbon present in organisms left this equilibrium-system is possible. This is usually equivalent to dating the death of the organism. The process of measuring the radioactivity, and hence the age of a carbon specimen, involves washing, shielding from other sources of radiation, transformation of the carbon into powder or some carbon-containing gas, and measurement with a counter. Various dates of biological, geological, and archaeological interest have been obtained in this way. Those dates of objects of known age have given consistent results, helping thereby to confirm the validity of the method. Other dates, measuring objects up to 40,000 years old have been obtained, including trees felled in glacial readvance in Wisconsin (about 11,000 years ago), evidences of human activity in North America (10,000 years), charcoal paintings in Lascaux Cave (15,000 years), and confirmation of Mayan and Babylonian calendars.

ROBERT MACARTHUR

MICROBIOLOGY SEMINAR

February 14, 1957

STUDIES ON GERM-FREE ANIMALS. By James A. Reyniers, Department of Bacteriology, Lobund Institute, University of Notre Dame, South Bend, Indiana.

The interest in germ-free animals has been directed to studying germ-free life per se, as well as using germ-free animals as media for further investigation. The animals for these studies have included mammals of all varieties as well as birds and chickens. The former are delivered free of contamination by cesarean section, while the latter are obtained germ-free by chemical sterilization of their shells. Rearing and breeding these animals is no longer a problem, and colonies of chickens, rats, and mice have been propagated, germ-free, for several successive generations. The apparatus employed in these studies, once limited to simple churn jars and milk bottles, now has become elaborate units kept sterile by steam under pressure. The problem of transporting these units has been solved recently with the development of special air-conditioned vehicles.

With the development of the techniques and apparatus required for rearing and breeding germ-free animals, studies have been conducted utilizing these animals for investigation of other problems. Thus, the rôle of certain enterococci in the formation of dental caries has been elucidated using germ-free rats. In another experiment, the relationship of coliform bacteria to *Entamoeba histolytica* has been clarified with respect to the

latter's ability to form lesions and cause disease. Other problems including hemorrhagic shock, radiation sickness, and animal immunity have been studied using germ-free animals. Finally, germ-free animals have been employed in the study of tumor formation. While spontaneous tumors have never been observed in these animals, injection of methylcholanthrene has produced very large tumors in chickens. Subsequent tissue filtrates of these tumors have indicated the presence of virus particles which appear to be responsible for both tumor genesis and tissue wasting.

S. I. H.

ZOOLOGICAL JOURNAL CLUB

February 20, 1957

REGULATION AND REGENERATION. By Richard J. Goss, Brown University, Providence, Rhode Island.

In the taste barbule of a catfish, the blastema tissue comes from the perichondrial cells of the interior cartilage rod. When the rod is removed, there is no regeneration. If three rods are added, only one terminal rod is reproduced when the tip is amputated.

If the end of a fish fin ray is amputated, an undifferentiated blastema elongates to form a full ray. No regeneration takes place when the dorsal fin ray is entirely removed since only osteoblasts can form dermal bone. If a fin ray is added in the inter-radial region, an extra structure is added by the supernumerary bone.

Removal of an amphibian ulna, and subsequent amputation of the hand, results in the regeneration of an ulna and normal digits. If the bone is replaced on the opposite side, an additional ulna is still produced as well as a normal digital extremity. When the ulna and skin are removed, only two digits and no ulna are produced in the regenerate.

With total x-ray irradiation (2,000 roentgens), no regeneration takes place following amputation. Irradiation of only one lateral half of the limb gives a half regenerate. Further experiments with x-rays and replacement of scrambled mesoderm in undisturbed skin indicate that the least disturbed tissue takes the major rôle in regulation although x-radiation of the mesoderm alone interferes with regulation by the skin. Any x-ray recovery is due to migration of nonirradiated cells and not to recovery of the cells themselves.

G. E. W. 3RD

ZOOLOGICAL JOURNAL CLUB

February 27, 1957

FUNCTIONS OF THE NOCTUID TYMPANUM. By Asher Treat, City College of New York, New York.

Noctuid moths escape bats at night. When the bat approaches within one meter, the moth changes from its regular zig-zag to a faster random flight. The bats, therefore, frequently miss their prey. This behavior has been

thought to be associated with sound reception by the tympanic membranes located on the moth's metathorax above the leg articulation. Experiments in destroying the membranes are unsuccessful, however, since damaged moths will not fly.

Impulses recorded from the tympanic nerve show that the lower intensity threshold and reaction time of the moth are about the same as for the human. Intensity is relayed to the pterothoracic ganglion by a delay in impulses. The nerves respond to 240 kilocycles/second, higher than any other known "ear," but it is impossible to tell if this is a pure tone. Greatest sensitivity, however, is from 15-60 cycles/second. Noctuids appear to be tone deaf; nerve responses do not distinguish between sonic and ultrasonic sounds. This is not surprising since they have only two sensory cells. The moths cannot localize the bat but are merely stimulated to go into random dodging flight.

G. E. W. 3RD

RUSSELL H. CHITTENDEN LECTURE IN BIOCHEMISTRY

February 27, 1957

AMINO ACID ACTIVATION AND PROTEIN SYNTHESIS. By Fritz Lipmann, Biochemical Laboratory, Massachusetts General Hospital, Boston, Massachusetts.

Compounds are activated by nucleotide triphosphates either by joining with the split terminal phosphate in a kinase reaction, thus eliminating the nucleotide diphosphate, or by joining with the nucleotide monophosphate, eliminating pyrophosphate. Carbamyl and succinyl activation represent the first type of reaction, sulfuryl and choline and amino acids the second. The latter has been studied by trapping the amino acid with high concentrations of hydroxylamine. Beef pancreas has been shown to have a greater capacity than liver to activate most amino acids, notably tryptophane. Analogues of tryptophane are also activated by the enzyme system, but tryptophane has the highest affinity. Some analogues of tryptophane are not activated, and these inhibit the activation of tryptophane. Those compounds that were not activated were not incorporated into proteins. It was postulated that after activation, amino acids are taken over by a carrier, probably a polynucleotide, before being assembled into proteins.

J. P. G.

ZOOLOGICAL JOURNAL CLUB

March 5, 1957

THE CLASSIFICATION OF NATURAL COMMUNITIES. By R. Whittaker, Brooklyn College, Brooklyn, New York.

There have been four principal approaches to the problem of classifying natural communities. The basis for these has been physiognomy, dominant species, dominant strata, or characteristic species (forms with a narrow distribution which are characteristic of a particular association). The first two

of these are most used by British and American plant ecologists, the third principally by the Russians and Scandinavians, while the last is much used in middle and southern Europe. In part, this usage pattern reflects the differences in the biotypes of these regions.

A detailed analysis of the vegetation at different altitudes of the Siskiyo Range of northwestern United States was carried out in order to determine whether any of these approaches allowed one to detect sharp discontinuities between communities. As a result, it was possible to demonstrate that species are distributed along continuous environmental gradients and that different species do not show concordant variations in abundance but are heterogeneously distributed. This is the obvious outcome of each species being distributed in accord with its own physiologic needs. The result is that individuals, not groups, vary in abundance, and the community boundaries are not sharp.

Many different methods of classifying natural communities can be used, the choice of any one method being determined by the needs of the particular ecologist. The important point to be stressed is that the available data indicate that no unit inherent to natural communities exists. The discontinuity which the plant ecologist finds is imposed by his own methods.

P. K.

ZOOLOGICAL JOURNAL CLUB

March 8, 1957

CHANGES IN THE FINE STRUCTURE OF MITOCHONDRIA DURING SPERMATOGENESIS. By Jerome S. Kaye, Zoology Department, Columbia University, New York, New York.

Phase contrast and electron microscope studies of the prosobranch snail, *Viviparus*, reveal that the internal structure of mitochondria does not break down during spermatogenesis. Cristae are visible throughout the whole process of nebenkern formation although the over-all shape of the mitochondria undergoes condensation, elongation, and coiling. During first metaphase, the eight mitochondria join end to end into four elongated rods and line up parallel to the spindle in the idiozome region. The rods break and segregate during the first division but divide autonomously at second anaphase. They elongate further and coil into a helix about the axial filament in sperm formation. A rearrangement of the internal structure parallels the outer morphological changes. When the mitochondria are 3.5 μ long, the cristae lie irregularly parallel to the long axis of the mitochondria. When the mitochondria elongate, the cristae are found at right angles to the long axis. They lie in eight rows in the mitochondria of the spermatid. Electron microscope pictures are clear enough to permit counting and calculation of the number of cristae per mitochondrium (1100). The total area is 60 μ^2. Since the mature nebenkern is a perfect helix, the number of cristae (4100) and the total cristae area (52 μ^2) can be calculated. The two areal figures are in good statistical agreement. Therefore, there is no synthesis of

cristae material during spermatogenesis. The change in number of cristae may be due to folding.

The helical shape is flexible probably to permit freer movement during swimming of the sperm than the usual mitochondrial cylinder. Internal differentiation may be due to a change in the energy output of oxidative metabolism.

G. E. W. 3RD

YALE MEDICAL SOCIETY

March 11, 1957

IN VITRO STUDIES OF PROTEIN SYNTHESIS. By Melvin V. Simpson, John R. McLean, George Cohn, and Ira Brandt, Department of Physiological Chemistry.

Although the microsome fraction of cells are believed to be most active in incorporating amino acids into proteins, actively phosphorylating mitochondria in the presence of the subcellular soluble fraction also incorporate amino acids into proteins. Contamination of the mitochondrial fraction by microsomes was ruled out by two different methods. In one, microsomes heavily labeled with radioactive leucine were added to nonlabeled mitochondria and the mitochondria separated and noted not to be radioactive. Secondly, adenosine-diphosphate inhibited amino acid incorporation into the protein of the mitochondria and microsomes in a quantitatively different manner; and ribonuclease inhibited completely amino acid incorporation into the microsomal protein without inhibiting (perhaps even increasing) incorporation into mitochondrial protein.

J. P. G.

STUDIES ON THE MUTATION OF BACTERIA. By Henry P. Treffers, Akira Ghoda, Christian Lerche, Kaare Jyssum, Dorothy Alexander, and Florence Thanassi, Department of Microbiology.

Bacterial growth and the various characteristics of it are often unpredictable. Mutation and selection explain most of the changes which occur as, for example, alterations in nutrition and variations in susceptibility to antibiotics. Hence, mutation is studied, in particular, the hypothesis that the rates of mutation are themselves governed by a special mutator gene.

It has been noted that stock cultures differ in their mutation rates and that the mutator gene can be transferred into other stocks by recombination and induction. Stock *E. coli* cultures are irradiated with ultraviolet light and blocks are produced in various stages of the metabolism of the sulfur-containing amino acids. Reversion rates are measured at each block. Various stock cultures are then crossed by recombination or transduction, and they are scored. In many cases increases in the rates of reversion may be seen, and this is taken as evidence that the mutator gene has actually been transferred into this stock.

A screening test is used to determine the presence of mutants and the following is an example. Full-grown cultures of *E. coli* are plated on

streptomycin agar. Normal cultures yield from none to a few colonies. Resistant cultures are overgrown, while mutant ones yield from twenty to one hundred colonies. The latter is repeated since occasional normals will do this, although not consistently. There is a wide variety of properties which may be tested in the same manner.

This basic plan has brought several problems to the fore. First, what are the mechanisms of mutator gene action? Stocks whose reversion rates are fifty times apart nevertheless revert at identical rates (three times ten to the minus seven) in its presence. Also, the nature of the influences of media is not known, although it is postulated that the gene may fill a metabolic defect. Second, why does the gene have no influence under certain circumstances? Third, what are the details of the reversion to repair a metabolic lack? Last, screening of two hundred random clinical coli samples showed a 7 per cent incidence of the phenomenon. What is the distribution of the mutator gene among other bacterial species such as *Staphylococci?* Research is now being carried on along these lines.

J. A. O'N.

EDGAR ALLEN MEMORIAL LECTURE

March 11, 1952

BIOLOGICAL MECHANISMS FOR ENVIRONMENTAL CARCINOGENIC AGENTS. By Paul Kotin, Associate Professor of Pathology, University of Southern California, Los Angeles, California.

Combustion products of gasoline and diesel fuel, in addition to certain atmospheric aliphatic compounds derived from gasoline, have been indicted as contributors to the increasing incidence of lung cancer. Experiments with mice show that these compounds are not only carcinogens, but that they damage bronchial epithelium, rendering it more receptive to invasion by particles carrying carcinogenic substances. Cigarette smoke has a similar effect on the epithelium, increasing uptake of carcinogens by the lung. It is possible that the high incidence of lung carcinoma in the male is related to hormonal factors which influence the biological activity of these particles.

L. S.

ZOOLOGICAL JOURNAL CLUB

March 13, 1957

SOME PROPERTIES OF STREAMING PROTOPLASM IN THE AMOEBA. By Robert D. Allen, Department of Biology, Princeton University, Princeton, New Jersey.

Specimens of *Amoeba dubia* which had ingested spheres of different materials were examined through a horizontal microscope with a vertical revolving stage in order to determine some of the properties of their cytoplasm. Spheres of iron carbonyl (2-8 μ), gold (7-25 μ), and mercury (2-23 μ) were used. Small spheres are held rigidly in the part of the cyto-

plasm which has classically been regarded as the liquid "sol." A sphere exerting a shearing force up to seven dynes is held by the endoplasm; a "plug force" stop-and-go type of movement is shown with forces up to fourteen dynes; higher than that figure, however, the spheres drop with gravity to the outer gel layer. Spheres of less than seven dynes may be moved "up hill by the protoplasmic streaming at the same speed as other intracellular granules."

Various agents will break the "gel" of the endoplasm. Under a pressure of 6,000 pounds, an iron sphere is not held but dropped in three seconds. Similar results obtain from putting the Amoeba in ribonuclease and from mechanical agitation.

In a giant amoeba, *Chaos chaos,* confined in a capillary tube and sealed with oil, cytoplasmic streaming is normal. If the ends of the tube are broken, the cell membrane and the outer gel material shrivel and are no longer visible. No differentiation is seen in the resulting "naked protoplasm," but streaming continues for up to 72 minutes. At first the geometry of the streaming is identical with the "core streaming" of the normal animal except that a distinct backflow is seen at the periphery. After ten minutes, core streaming breaks down and up to six constellation streams are observed moving either backwards or forwards with reversible contractions. If calcium is present in the culture, contractions are irreversible, flowing ceases, and vacuoles form, similar to breakage injury vacuoles. In this multiple streaming, gold spheres of 20 μ do not fall. Finally a subdivision of the streams into two-directional flows is seen following a build-up of tension.

Numerous combinations of nonliving materials in various physical environments were tried in an attempt to duplicate amoeboid streaming, but none reproduced the actions seen in living Amoeba.

Possible conclusions are that amoeboid cytoplasm has labile structure and the visible flow which was formerly thought to be passive has the ability to stream of its own accord.

G. E. W. 3RD

LEON E. SAMPLE LECTURE

March 20, 1957

HUMORAL AND HORMONAL FACTORS IN WOUND HEALING. By J. Englebert Dunphy, Professor of Surgery, Harvard Medical School, Boston, Massachusetts.

Wound healing, or tissue repair, is conceived of having several components: (1) epithelialization; (2) granulation tissue formation; (3) healing in special tissues. Granulation tissue is regarded as the "wound blastema." It represents a special phase of healing beginning at the day of injury and proceeding to cicatrization. The phenomenon of wound contraction is related to granulation tissue but is not dependent upon collagen formation. Contraction represents an example of tissue morpholaxis in that the wound is remodeled without new tissue formation.

Based upon biochemical analyses and histochemical staining methods two phases of granulation tissue production are recognized. One, a productive or substrate phase beginning within a few hours of wounding and lasting for five or six days, and two, a collagen phase which begins about the sixth day and lasts until healing is complete. Various stages of this process have been shown to be influenced by such factors as ascorbic acid, stress, exogenous sources of protein, and adrenocortical steroids. Qualitative study of wound repair and the effects of various factors on the process have been approached by tensile strength and more recently by histochemical staining techniques and biochemical quantitative analysis. By implanting a polyvinyl sponge in healing tissue, it is possible to separate newly formed from pre-existing tissue. This technique provides an opportunity for microscopic study of the stages of a granulating wound in the matrix of the sponge. The main drawback is the difficulty in standardizing the technique in humans although in animals this is less of a problem. Over-all results have agreed with the previous tensile strength studies.

Using this technique, studies of collagen concentration in granulation tissue in normal rats, adrenalectomized rats, both normal and adrenalectomized rats treated with cortisone were carried out. These showed that cortisone administered to the normal animal reduces the rate of collagen formation in a wound. Administered to an adrenalectomized animal cortisone improves the rate of collagen formation to slightly below the normal curve.

Further studies have explored the concentration of mucopolysaccarides in relation to the concentration of collagen in the wound. It has been found that the mucopolysaccarides rise over the first four days and then fall as the collagen concentration rises, implying that the collagen formation may depend upon a substrate of mucopolysaccarides providing that ascorbic acid is available in adequate amounts. Protein starvation experiments have shown that the phase of mucopolysaccaride formation is slowed as is the subsequent collagen formation. Methionine supplied to the protein starved rat will enhance mucopolysaccaride and collagen formation to near normal levels.

It is postulated that there is a wound blastema of "organ of tissue repair" which exchanges hormones and metabolites with other organs and tissues and represents the response to those accelerative and decelerative factors which are presently known. The ultimate aim of these studies is to define the biochemical, physiological, and anatomical aspects of the wound blastema not only as a means of influencing repair in surgical wounds, but also to understand repair as a basis phenomenon common to all injury and disease.

R. S. N.

THE FOSSIL EVIDENCE FOR HUMAN EVOLUTION. An Introduction to the Study of Paleoanthropology. By W. E. LeGros Clark. The Scientist's Library. Chicago, University of Chicago Press, 1955. 181 pp., 20 figs. $6.00.

Remains of ancient man and his immediate ancestors are among the rarest of fossils and usually are extremely fragmentary as well. Those that have been discovered have received such intensive study, however, that the publications on fossil man and human evolution rival in numbers those devoted to all other fossil vertebrates combined. New discoveries in a field where data are so scarce inevitably require frequent revisions of general conclusions, such as are apt to be quoted in textbooks. Several new interpretations of the human family tree have been necessary during the past decade, and sharply divergent views have been expressed on certain points by well-known scholars. For these reasons Professor LeGros Clark's concise, scholarly, and extremely critical review of the present state of knowledge of human evolution is a most valuable reference for all interested in this subject.

The serious student who sets out to master this small volume will find much to think about. Every zoologist would profit by careful study of the opening chapter, an essay on the problems of morphology, taxonomy, and phylogeny, and the need for precise terminology in discussions of this sort. The general reader, if he can persevere through its 47 pages of critical analysis, would be well prepared to evaluate statements about the significance of various features of ancient human skeletons for their relationships to other fossil and living types.

A conservative classification and nomenclature is advocated for the family Hominidae, only three genera, *Homo, Pithecanthropus, and Australopithecus* being recognized. (The book was written prior to Hürzeler's demonstration that the late Miocene *Oreopithecus* is a hominid). It will surprise many to learn that the most distinctive feature of the Hominidae is not large brain size, which was only acquired in the latest stages of human evolution, but rather the development of erect, bipedal locomotion, in contrast to the brachiating specialization of the Pongidae (great apes). Distinctive features of the hominid dentition likewise appeared early, which is fortunate for interpreting the fragmentary fossils, most of which are teeth or jaws.

Commencing with modern man, significant fossil types are considered in order of increasing age. Neanderthal man is a localized, isolated group which inhabited Europe during the last glaciation. Still more ancient men in Europe and elsewhere in the Old World are not separable from *Homo sapiens*, although they have some primitive features. Some of the "neanderthaloid" populations such as Solo man, Rhodesian man, and Mt. Carmel man may provide the basis for the independent evolutionary differentiation of some of the major races of present-day mankind, but the author warns that anatomical evidence is inadequate to demonstrate racial differentiation before the end of the Pleistocene.

The middle and possibly early Pleistocene genus *Pithecanthropus* includes not only the famous Java "Ape Man" but also the Chinese fossils originally called *Sinanthropus*. Considerable attention is paid to the now abundant evidence that these are closely similar to *Homo* in a large number of com-

plex anatomical features, and differ from modern man principally in their smaller brain capacity. Still more detailed analysis of the recently discovered South African fossils, of early Pleistocene age, convincingly demonstrates that *Australopithecus,* and numerous other named types whose generic distinctness is questioned, are clearly erect hominids whose brain size in some individuals did not exceed that of the larger great apes. A final brief chapter treats the scanty evidence for the separation of the Hominidae from the Pongidae during the Pliocene or late Miocene. Recent discoveries probably will require revision of some of the points made here.

Professor LeGros Clark writes with economy of words and with great precision, yet with a flowing command of the English language rare in a scientist. He has brought the experience of many years of research on living and fossil Primates to bear upon the theme of man's physical evolution, and distilled the essential data and conclusions from a formidable mass of specialized publication.

JOSEPH T. GREGORY

LIFE AND MIND. By Edmund Ware Sinnott. Yellow Springs, Ohio, The Antioch Press, 1956. 29 pp. $.50.

In this, the Second Founders Day Lecture at Antioch College, Edmund Ware Sinnott, the distinguished geneticist, faces the age-old problem of the relationship between mind and body. After calling attention to the fact that for science the body of man, as well as other living systems, "is a physico-chemical system which manifests the operation of those laws that are found in lifeless systems," he notes that the continued existence of every living organism is dependent upon the precise ordering of all the chemical entities which make up the soma, though these are in constant flux.

It is clear, however, that for many people this cannot be the whole answer, for many have an intuitive feeling that there are in man some characteristics which cannot be defined in terms of physico-chemical laws. This is another way of asserting that, although the body is a physical-chemical machine, it possesses other characters loosely called mind, or soul, or spirit which, in some obscure fashion, manipulate the machine. Thus we have on one hand materialism, and on the other, a mixture of the material with the spiritual. The scientist can only work with the basic assumption that there is "uniformity and dependability of natural law everywhere, even in man."

In contrast, many men are quite convinced that to be alive is to be free—free to make choices, free to seek goals, to satisfy desires, and to accept or reject moral law. For Professor Sinnott, this second position requires an adequate answer to the question, "What is mind?" It is clear enough, as Sir Charles Sherrington pointed out, that we have no measure of mind except in the way it expresses itself in behavior. So far as we know, it cannot be separated from the nervous system. The position that Dr. Sinnott takes—that every living system in the course of growth and development exhibits astonishing self-regulation and adaptive behavior—implies that in the living system there is some force or forces which maintain the characteristic pattern of organization in the face of constantly changing chem-

istry. Because we cannot define these forces, philosophers and saints from Aristotle to Driesch have, over the years, assigned names such as "final cause," "the élan vitale," "the entelechy," "the life principle" to this organizing principle. Since we cannot measure this principle by modern techniques, scientists prefer to ignore its existence, although they must necessarily admit its presence.

Professor Sinnott makes the very important plea that no one can afford to be dogmatic about this problem. The true scientist is a modest person. He knows full well that he does not know all the answers. There is no reason to believe that we know all the laws of nature, although we may be able to define some of them. New techniques may reveal new laws so that what cannot be defined at present may some day be made clear. In the meantime, it can be argued that mind bears the same relation to behavior as the organizing self-regulatory forces do to growth and development. Moreover, since it is generally accepted that every living organism is something more than the sum of its parts, so Man is not just a physico-chemical system but a marvellous combination of a machine with the qualitative attributes which we call the mind.

H. S. BURR

HANDBOOK OF BIOLOGICAL DATA. William S. Spector, Ed. Philadelphia, W. B. Saunders Company, 1956. 584 pp. $7.50.

Under the auspices of the National Research Council, 17,000 scientists cooperated to tabulate established biological data in handbook form, analogous to the *Handbook of Chemistry and Physics*. This is only one of a series of handbooks published or in the process of publication; the others are more detailed and specialized and include *Standard Values in Blood, Standard Values in Nutrition and Metabolism,* and *Handbook of Toxicology*. The 445 tables presented here have been divided into ten sections: biochemical and biophysical characteristics; genetics, cytogenetics, and reproduction; development and morphology; nutrition, digestion, and metabolism; respiration and circulation; other physiological activities and performances; biologically active compounds; environment and survival; symbiosis and parasitism; ecology and biogeography. An appendix containing taxonomic, conversion, and other, more prosaic, tables is included along with a 39-page subject index.

There is a table to engage everyone's interest. The composition of lymph or the electrophoretic analysis of plasma proteins of most organisms can be compared at a glance; the pH of standard solutions, cells, tissues, organs, cell sap; the amino acid composition of proteins and micro-organisms; the breeding habits, cerebrospinal fluid composition, growth rates, and blood counts of all organisms; the metabolic pathways for almost everything; antimetabolites and other drugs; organ function tests; radiation and isotopes; and much about everything else. It is a truly remarkable compendium of little known facts. Scientists in the most divergent fields can find a table pertinent to their work. There is some question as to whether this Handbook is useful, as the more detailed monographs mentioned above are, for

it is such a potpourri. However, its very scope is intriguing and makes the book enticing to thumb through. One cannot say that no biologist's bookshelf should be without it, but whether it yields a relevant fact or satisfies idle curiosity or simple pedantry, the book is worth the price.

J. P. G.

ESSENTIALS OF HISTOLOGY. By M. M. Hoskins and G. Bevelander. St. Louis, C. V. Mosby Co., 1956. v, 254 pp. $4.00.

The beginning student of histology is frequently confused by the increasing burden of detail that is incorporated into the more encyclopedic texts. For such students the more condensed treatment available in this book will prove useful since it provides, in skeleton form, the essentials of the subject. In this sense the present (third) edition of this work fills a real need in providing the student with a frame of reference. The fact that the scope of this book can provide only a foundation is clearly recognized by the authors who rightly suggest that the superstructure must be added from other sources.

The scope of the third edition is little different from that of its predecessors. Most of the illustrations are line drawings which serve admirably to emphasize the salient characteristics of each tissue and organ. The diagnostic tables of previous editions have been retained and are of some value. The absence of bibliographical material, while in keeping with the aim of the book, underlines the necessity, in all but the most abbreviated courses, of recourse, in addition, to more detailed treatments.

The present reviewer must note one drawback in the present revision. At several points it fails to reflect fairly recent advances in microscopic anatomy. For example, despite recent advances in histochemistry and electron microscopy, these fields are not mentioned in the text, the latter field being represented by a lone electron micrograph. Again, in the section on liver no use is made of the newer data of Elias and, as a consequence, the figures belie the text. While the preface promises to ". . . borrow material from other disciplines . . . in several instances to correlate structure and function," the text does so to a very limited extent. No matter how brief a textbook is, it should be up to date and should reflect the current status of the field.

With this one reservation the third edition of *Essentials of Histology* will continue to serve well the beginning student in search of orientation.

ALFRED J. COULOMBRE

BLAKISTON'S NEW GOULD MEDICAL DICTIONARY, 2d. ed. Normand L. Hoerr and Arthur Osol, Eds. New York, The Blakiston Division, McGraw-Hill Book Co., 1956. xxvi, 1463 pp. 252 illus., 129 in color. $11.50.

The present edition is a comprehensive dictionary of the terms used in the numerous branches of medicine and closely related sciences, including radiobiophysics, chemistry, dentistry, pharmacology, veterinary medicine, zoology, botany, and forensic medicine. The excellence of the second edition is

the result of the efforts of Drs. N. L. Hoerr, A. Osol, S. W. Chase, Carl F. Francis, and the co-operation of eighty-eight contributors.

A special effort has been made to include some of the newer terms from the vocabularies of thoracic surgeons, psychiatrists, and tumor investigators. Oncologic terms have been increased by at least two hundred. In microbiology, the names of genera have been modernized to agree with the latest terminology. Pharmacologists will welcome this edition, especially since it includes and defines the numerous drugs described in *The Pharmacopeia of the U.S.A.* (*U.S. Pharmacopeia*) Fifteenth Revision (1955), and in the *New and Non-official Remedies* (1955).

Anatomists will find the present edition particularly helpful because it contains the BNA (Basal Nomina Anatomica) terms with modifications and those BR (British Revision) terms that differ significantly from them. Also included are parts of the old terminology (OT) and the recent modifications suggested by the International Nomenclature Commission. From this reviewer's appraisal, it appears that the second edition is much more extensive than the so-called "first" edition (a revision of Gould's Medical Dictionary, 5th ed., 1941). The definitions are accurate, and in many instances several definitions are provided where necessary. The 1956 edition includes the new terms and usages which evolved with the myriad medical advances during the first half of the twentieth century.

Included are 45 illustrative plates, several of which are in color. The illustrations are well done and correctly labeled. They will be useful to the student of human biology and the practitioner alike.

The appendix consists of 116 pages of tables of information ranging from anatomy (arteries, bones, joints and ligaments, muscles, nerves, and veins), blood constituents, chemical elements, enzymes, hormones, and isotopes to prescription writing, vitamins, and weights and measures.

This dictionary is a useful one; the print is clear, and the paper is of excellent quality. The excellence of the first edition is richly enhanced, and this reviewer is confident that the present edition will be useful to students, medical practitioners, writers, and secretaries. Furthermore, it is the most recent dictionary, and its large number of words, illustrations, and tables will appeal to those who may wish to purchase a medical dictionary for the first time or those who may wish the latest edition of a medical dictionary.

JOSEPH T. VELARDO

THE HISTORICAL BACKGROUND OF CHEMISTRY. By Henry M. Leicester. New York, John Wiley and Sons, Inc., 1956. vi, 260 pp. $6.00.

In this slim volume, Professor Leicester has set himself the Herculean task of presenting the history of chemistry from prehistoric times to the present. While it is inevitable that such a survey will necessarily skimp in some places or ignore whole areas, Professor Leicester's work stands up, on the whole, as a literate and accurate account of man's pursuit of knowledge of the intimate processes of nature.

There are two main dangers which the author of any survey must face; description tends to take the place of analysis and, in an attempt to mete

out the proper measure of historical fame to all participants, the narrative may turn into a simple catalogue of names and dates. Both of these elements exist in *The Historical Background of Chemistry.* As Professor Leicester approaches the modern scene, he has space only to march his characters rapidly across the stage and off into the wings. Theory and theorists come in a somewhat bewildering sequence. The work is saved by the fact that Professor Leicester does stop and linger over crucial steps so that while discussions of details may be superficial, the total perspective remains clear and the main lines of chemical development are lucidly developed.

Professor Leicester's ability in threading through the maze of the history of nineteenth and twentieth century chemistry serves to underline his relative weakness in ancient science. Here he has relied upon the somewhat unreliable Abel Rey and J. Burnet and tends to go astray. In spite of the recent work of O. Neugebauer, for example, he repeats the old legend of Babylonian astrology and Eastern mysticism.

One final and pedantic note should be added. Professor Leicester gives his references as a chemist, citing only author and the journal in which articles have appeared but the titles of these articles no where are cited. This, and the fact that there is no bibliography, makes it difficult to use this book for bibliographical purposes. Since Professor Leicester is fully aware of the most recent literature in the history of chemistry and since he also refers constantly to the original papers of the chemists he mentions, he might have been less reticent and passed this information on in full to the reader.

The Historical Background of Chemistry is a valuable and useful study. It should serve equally well as a guide to the chemist and to the historian.

L. PEARCE WILLIAMS

BIOCHEMICAL INDIVIDUALITY. The Basis for the Genetotrophic Concept. By Roger J. Williams. New York, John Wiley and Sons, Inc. xiii, 214 pp. $5.75.

One of man's most important and impressive characteristics is his individuality. The fact that such individuality is characteristic of all organisms has long been recognized by biologists and constitutes the basis of current evolutionary theory. Implicit in such a concept is the acknowledgement of variance. In an attempt to emphasize the significance and meaning of normal variance, Dr. Williams has written in a clear and lucid style an interesting and thought-provoking little book. Not only can it be recommended to those in the field of medicine and psychiatry, but also to students in all phases of biological science, particularly to those in applied biology. Its implications, however, extend over a much wider range which includes anthropology, psychology, child development, education, and even religion, law, and politics.

The book is primarily devoted to presenting evidence that inter-individual variability does indeed exist on many levels, that these normal variations fall within an exceedingly wide range, and that this variability has a genetic

basis. Hence, material is compiled citing morphological and biochemical evidence indicating that each individual has a characteristic morphology and physiology, and that normal inter-individual differences are exceedingly great when specific items are studied. Among the topics discussed are variations between individuals in specific enzyme efficiencies, endocrine activities, excretory patterns, drug responses, and various nutritional requirements.

On the basis of this evidence, the author then presents his genetrophic approach in which he states his belief that nutrition applied with due concern for individual genetic variations, which may be large, may offer the solution of many baffling health problems. By virtue of such an approach, Dr. Williams directs attention to the need in human biology and medicine for more emphasis to be placed on variability and individuality at the physiological and biochemical level. He presents his thesis in the form of a challenge to further research in the area of individual nutritional needs. The implications of such an approach for medicine, psychiatry, dentistry, and the biological sciences are discussed.

Although the data assembled are in no way encyclopedic, the substance of the book is none the less valuable since this is the first attempt to collect whatever information exists on the subject of inter-individual variability. As such, then, it offers fresh insights to all those working in the fields related to biological sciences.

RUTH H. BELOFF

POLYSACCHARIDES IN BIOLOGY. Transactions of the First Conference, April 27, 28, 29, 1955. George F. Springer, Ed. New York, The Josiah Macy, Jr. Foundation, 1956. vii, 271 pp. $5.00.

The Josiah Macy Foundation and Dr. Springer convened an eminent group of scientists to begin this new conference series. M. L. Wolfrom, Karl Meyer, M. Heidelberger, and W. T. J. Morgan, together with many noted contributors, discuss the four topics: Nomenclature (20 pages), Problems of Classification (18 pages), Bacterial Polysaccharides (83 pages), and Blood Group Substances (101 pages).

In the first chapter, Wolfrom discusses some of the rules that the American Chemical Society Nomenclature Committee has evolved over the last five years concerning naming of carbohydrates. Next, Karl Meyer discusses his most recent classification scheme for the hexosamine or "muco-" compounds. In view of the wide gap in our knowledge of the structure and function of these compounds as well as their endless variety, this is a formidable task. Those confused by the terms "mucoid" and "mucoprotein" and those with biological material to classify will find guidance here. Still unclear, however, is the term "glycoprotein" at least from the sugar chemist's viewpoint.

The final two chapters are the main business of the book. Here the reader can get a realistic look at some current problems in polysaccharide chemistry. Of special interest is the wide use made of the old Landsteiner hapten inhibition technique to determine the nature of the immunochemically active

sites on the polysaccharide using simple sugars as prospective inhibitors. Although much discussion is given to this topic, a really critical evaluation of the method as it is applied would have been welcome.

As if overcome by long hours of work in purifying and assaying, the members of the conference several times lapse into rather unrewarding philosophical speculation on the question of whether biological macromolecules are ever actually homogenous in the micro sense. Perhaps all that could have been said on this topic without being definitive was said here. Since almost all of the data presented in the book can be readily found elsewhere, and often in better organized form, it would seem that the most important function served by this volume will be its occasional critical discussion of techniques and interpretations of data.

IRWIN A. ROSE

PAPER ELECTROPHORESIS. G. E. W. Wolstenholme and E. C. P. Millar, Eds. Boston, Little Brown and Co., 1956. xii, 224 pp. $6.75.

This book records the proceedings of a symposium on paper electrophoresis sponsored by the Ciba Foundation and held in London, July 1955. The meeting was small and select, attended by 22 active workers in the field from all over the world. The chief object of this book is to make available to the general scientific public the results and discussions which transpired. Some 15 papers and short communications, interspersed with the full discussions which followed the presentation of the papers, make up the book.

Since about 1948, paper electrophoresis has undergone an enormous and rapid development. Its principal function has been to provide clinical medicine with a relatively inexpensive method for performing routine electrophoretic analyses of serum proteins, although its usefulness as a unique research tool is amply indicated in several articles in the book. The technique, however, is deceptively simple in appearance. In fact, both theoretically and experimentally, paper electrophoresis is more difficult than moving-boundary electrophoresis, from the point of view of obtaining absolute and reproducible quantitative results. The paradoxical situation therefore exists that the more difficult technique is by and large in the hands of less critical and research-minded scientific personnel, and this situation requires the exercise of caution.

Many of the articles in this book, by carefully analyzing various aspects of paper electrophoresis, bring this situation into clear focus. An interesting, if brief, paper by H. Svensson enters into the physical chemical aspects of the technique and their relation to the design of suitable apparatus. This paper makes clear how much more complicated electrophoresis is in paper than in free solution. Other articles by E. M. Crook, H. J. McDonald, and N. H. Martin deal critically with the analysis of paper electrograms, including problems of staining, elution analysis, direct optical scanning, area resolution, and other important analytical aspects. The discussions following the papers are very useful in bringing up many practical questions concerning the technique, and in dispelling some misconceptions held even by some of the experts attending the symposium. In addition, there are several papers

dealing with specific applications of paper electrophoresis to problems of diagnosis of disease, to serum analyses, to the separation of the closely related kinds of human hemoglobins, and to lipoproteins.

A book like this is not meant to, and never can, take the place of a well-organized, methodical reference book on a particular technique. While the informal meeting of a small group of similarly directed scientists is an extremely valuable method for exchanging ideas, it suffers by its very nature in having too narrow a participation and point of view. For example, paper electrophoresis certainly has its theoretical foundations in two fields: moving-boundary electrophoresis, and the general physical chemistry of proteins (particularly where subjects such as protein-ion and protein-dye interactions are concerned). Yet only one participant in the symposium, Svensson, has been pre-eminent in these fields. Despite such shortcomings, however, this book should be of considerable value to practitioners of paper electrophoresis.

S. J. SINGER

RECENT PROGRESS IN HORMONE RESEARCH. Gregory Pincus, Ed. Vol. 12. New York, Academic Press, Inc., 1956. 431 pp. $10.

This volume, the Proceedings of the 12th Laurentian Hormone Conference (September 1955), represents broad and yet detailed consideration of many of the frontiers of endocrine investigation. The participants include many of the leaders in endocrine research; their contributions entail fundamental biochemical investigations as well as clinical studies. Among the most valuable aspects of the *Proceedings* is the recording of the scintillating and challenging discussion periods which followed the formal papers.

Part I. Hormone Biosynthesis and Metabolism, analyses in detail recent work in this area, individual papers being devoted to thyroid, adrenal medullary, and steroid hormones.

Part II, Hormones and Metabolism, includes contributions on the influence of steroids on human cerebral metabolism, on the relationship of aldosterone to human salt and water balance, together with a fascinating paper by Krahl on the influence of hormones on peptide formation in animal cells.

Part III, Pituitary Hormones, consists of a detailed review by Albert of the technical and interpretive problems entailed in human urinary gonadotropin assay, of a review by Lerner and Takahashi of hormonal control of melanin pigmentation, a field to which they have contributed so much, together with an analysis by Escamilla of the various pituitary syndromes found in man.

Part IV, Sex Hormones, includes an analysis of the rôle of the male sex hormone in reproduction, a clinical study of testicular hormone production, and an experimental study of the effects of ovarian steroids on myometrial function.

These *Proceedings* constitute a veritable storehouse both of established facts and of current pathways of investigation in the endocrine area.

PAUL MANDELSTAM

GLAUCOMA. Transactions of the First Conference, December 5, 6, 7, 1955. Frank W. Newell, Ed. New York, Josiah Macy, Jr. Foundation, 1956. 251 pp. $4.50.

Glaucoma contains the transactions of the first conference held in Princeton in 1955 and sponsored by the Josiah Macy, Jr. Foundation. This is one of more than twenty conferences selected to represent a multidiscipline approach in this case to the problem of glaucoma. It is an attempt to stimulate research and to promote effective communication across the departmental walls which tend to isolate the professions and specialties from one another. Clinical and research specialists in ophthalmology, internal medicine, psychiatry, and pharmacology made up the participants.

The conference was divided into three major divisions: I. A review of angle-closure glaucoma; II. Central control of intraocular pressure; and III. Resistance to aqueous outflow.

The first part on so-called angle-closure glaucoma concerns itself principally with the clinical problem of angle-closure glaucoma. The second and third center chiefly about related experimental problems.

This conference on glaucoma serves as an excellent example of the interchange of ideas and scientific progress that can be accomplished by teamwork. It resembles the approach used by ophthalmologists, pediatricians, and others in helping to understand and prevent retrolental fibroplasia. It can be said that the participants in the conference were in complete agreement that the increased tension of angle-closure, or narrow-angle glaucoma, is on the basis of the iris root blocking the flow of aqueous through the trabecula. They agree that a relative pupillary block is the primary basic mechanism involved. Of secondary importance is pupillary dilation which can have a varied rôle. At times it will loosen the peripheral iris enough to allow it to be pushed against the trabecula; at others it may bunch up sufficient iris into the angle to block outflow in its own right; and finally it may have the antagonistic rôle of breaking a pupillary block mechanism.

The actual events which trigger an attack are discussed and there is some disagreement on the physiology involved. In general, there is thought to be some central nervous system influence on these trigger mechanisms. It seems improbable that increases in inflow or outflow of aqueous are of much importance. It is possible that changes in the vascular bed of the choroid and ciliary body could be of sufficient volume to initiate an attack in a narrow-angle eye. Such changes in volume could be produced either acutely or chronically by psychological influences.

When an angle block by iris has occurred, the attack may be aborted by the miosis produced by exposure to light or by the miosis of the sleep mechanism. It is possible that the increased pressure could inhibit aqueous production, or the attendant pupillary dilation might break the pupillary block mechanism. Production of permanent synechias are dependent upon edema of the iris, and so may not occur in chronic narrow-angle eyes. Such eyes may have a decreased facility of aqueous outflow if the iris has rested against the trabecula for any length of time.

As to the central control of intraocular pressure to avoid premature speculation, since factual data are insufficient, no conclusions from the results of these experiments have been drawn with reference to the problem of glaucoma.

As to resistance to aqueous outflow, the central point of this discussion is the rôle of the trabecular mesh work which is considered as a tissue living off the aqueous humor. This mesh work may or may not be directly innervated. It may be influenced by the composition of the aqueous humor which in old age may be less nourishing. In turn the cells of the trabecular mesh work, the regulating properties, and the underlying connective tissue would suffer. It was felt that hyaluranic acid and hyaluronidase might play a part.

R. M. FASANELLA

LOW-FAT COOKERY. By E. S. Stead and G. K. Warren. New York, McGraw-Hill Book Company, Inc., 1956. 184 pp. $3.95.

This book concentrates almost exclusively on one nutrient—fat. It implies that in cases of overweight, heart disease, and diabetes low-fat menus are the answer, with total calories, protein, and carbohydrate relegated to minor rôles. Along this same line there is a somewhat misleading statement on the difference between fat foods and fattening foods.

A table of food composition is given for fat only, from which the values for the recipes are taken. The values given both in the tables and the recipes are carried to one decimal place. When foods are only measured, the accuracy implied by a calculation to one decimal place is not warranted. The units of description of the food items are not entirely consistent, e.g. value for one bread given in pounds while another is given for one slice.

In the comments on diabetes an inconsistency is noted in that one place it is stated: ". . . because in the diabetic person a constant intake of carbohydrate and protein at breakfast, lunch, and dinner is desirable," while in the next paragraph it states: "The patient need not be restricted in sugar and carbohydrate. . . ."

There is also a somewhat naïve explanation of why mineral oil should not be used.

The authors do not hesitate to use name brands of foods freely. Some of these are not universally available and may lead to expensive meals for people who will feel that they must have these particular foods.

No basic meal plan for a day is given but a week's menu each for two levels of fat intake are given. It is hoped that users of the book will not feel that they must confine themselves just to this one set of menus. A basic daily meal plan based on food groups would more likely insure variety as well as teach adequate nutrition for the lay person who must follow a low-fat regimen.

The book should be a good source of recipes for anyone for whom a low-fat diet has been prescribed. Instructions on such a regimen, however, should be given by a physician or dietitian.

DORIS JOHNSON

ADDITIONAL BOOKS RECEIVED

American Cancer Society, Inc. Committee on Growth. *Tenth Annual Report.* July 1954-June 1955. Washington 25, D. C., Division of Medical Sciences. National Academy of Sciences-National Research Council, 1956. xl, 325 pp.

Anatomical Atlas. By Maud Jepson, M.Sc. New York, Rinehart & Company, 1957. 24 pp. $0.75.

Ultramicro Methods for Clinical Laboratories. By Edwin M. Knights, Jr., M.D., Associate Pathologist, Director of Clinical Pathology, Roderick P. MacDonald, Ph.D., Director of Clinical Chemistry and Research Adviser and Jaan Ploompuu, Chief, Division of Ultramicro Chemistry and Research Associate. New York, Grune & Stratton, 1957. viii, 128 pp. $4.75.

Dissection Guides. By H. G. Q. Rowett, Lecturer in Zoology at Plymouth Technical College. I. *The Frog.* II. *The Dogfish.* III. *The Rat, with Notes on the Mouse.* IV. *The Rabbit.* V. *Invertebrates.* New York, Rinehart & Company, 1957. 63 pp., 62 pp., 64 pp., 32 pp., 56 pp. $0.95 each.

Advances in Virus Research. Edited by Kenneth M. Smith, Virus Research Unit, Molteno Institute, Cambridge, England, and Max A. Lauffer, Department of Biophysics, University of Pittsburgh, Pittsburgh, Pennsylvania. vol. IV. New York, Academic Press Inc., 1957. ix, 340 pp. $8.00.

The Genesis of the Rat Skeleton; a Laboratory Atlas. By Donald G. Walker and Zolton T. Wirtschafter. Springfield, Ill., Charles C Thomas, 1957. 59 pp. $7.50.

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references: 1. McDermott, W.: Ann. New York Acad. Sc. *65*:59 (Aug. 31) 1956. 2. Noyes, H. E.; Nagle, S. C., Jr.; Sanford, J. P., and Robbins, M. L.: Antibiotics & Chemother. *6*:450 (July) 1956.

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